CYCLIC ADENOSINE MONOPHOSPHATE-MEDIATED ACTIONS OF PROSTAGLANDINS IN THE CENTRAL NERVOUS SYSTEM AND UTERINE SMOOTH MUSCLE

BY

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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY

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DECLARATION

This is to certify that all the work reported in this thesis were carried out in the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria and the Department of Biomedical Sciences, University of Tampere, Finland. The work was done under the joint supervision of Professor C. Wambebe and Professor H. Vapaatalo. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

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CERTIFICATION

This thesis entitled *Cyclic Adenosine Monophosphate Mediated Actions of Protaglandins in the Central Nervous System and Uterine Smooth Muscle* by Gamaniel, Karniyus Shingu meets the regulations governing the award of degree of Doctor of Philosophy in Pharmacology, of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my daughter

ADATI (Jubilee)

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ABBREVIATIONS

Adenylate Cyclase	EC4.6.1.1; ATPpyrophosphate-lyase(cyclizing)
5'-AMP	Adenosine-5'-monophosphate
AA	Arachidonic acid
ATP	Adenosine-5'-triphosphate
CNS	Central nervous system
Contr.	Contraction
CSF	Cerebrospinal fluid
Cyclic AMP	Cyclic Adenosine-3',5'-monophosphate
Cyclic GMP	Cyclic Guanosine-3',5'-monophosphate
DA	Dopamine
EEG	Electroencephalogram
EPA	Eicosapentanoic acid
G.i.t.	Gastrointestinal tract
Guanylate Cyclase	EC4.6.1.2; GTPpyrophosphate-lyase(cyclizing)
I.c.v.	Intracerebroventricular
I.v.	Intravencus
I.p.	Intraperitoneal
IBMX	Isobutylmethylxanthine
Max.	Maximum
MLCK	Myosin light chain kinase
Min	Minutes
Minim.	Minimum

M	Molar
mV	Millivolt
µg	Microgram
µs	Microsecond
N	Normal
NA	Noradrenaline
Nmr	Nucleomagnetic resonance
PG	Prostaglandin
PGI_2	Prostacyclin
15-PGDH	15-Prostaglandin dehydrogenase
PTZ	Pentylenetetrazol
Pirox.	Piroxicam
PPS	Pulses per second
Phosphodiesterase	EC3.1.4.17; 3',5'-cyclic nucleotide-5'-nucleotidohydrolase
Protein Kinase	EC2.7.1.37; ATP protein phophotransferase
Relax.	Relaxation
STR	Strychnine
S.C.	Subcutaneous
SEM	Standard error of mean
Sec.	Seconds
T1/2	Half life
TX	Thromboxane
Vel.	Velocity
V	Volt
NSAID	Nonsteroidal anti-inflammatory drugs.

LIST OF PUBLICATIONS

(a) C. Wambebe, G. Osuide and K. Gamaniel (1984), "Influence of levodopa and 3,4-dihydroxyphenyl serine (DOPS) on strychnine induced seizures in mice", General Pharmacology, 15: 243-245.

(b) K. Gamaniel, S.L. Bodhankar and C. Wambebe (1986), "Effects of PGE_1 on the isolated rat uterus in the presence of verapamil", Paper presented at the the 6th International Conference on Prostaglandins (PG '86) in Florence (Italy), Abstract Book page 106.

(c) K. Gamaniel, H. Vapaatalo, C. Wambebe and H. Wuorela (1987), "Effects of iloprost, PGE_1 and prostacyclin on chemically and electrically induced seizures in mice", Prostaglandins, Leucotrienes and Essential Fatty Acids., In press.

(d) H. Wuorela, K. Gamaniel, T. Metsa-Ketela and H. Vapaatalo (1988), "Qualitative difference between the contractile effects of PGE_1 and oxytocin on the isolated rat uterus", Pharmacol. Toxicol. 60(III): 74

(e) K. Gamaniel, H. Wuorela, T. Metsa-Ketela and H. Vapaatalo (1988), "Influence of piroxicam on the contractile action of PGE, on the isolated rat uterus", Methods and Findings in Experimental and Clinical Pharmacology, 10: 493-496.

(f) K. Gamaniel, H. Vapaatalo and T. Metsa-Ketela, "A controlled study of the effects of dietary supplementation of mackerel oil on seizure susceptibility in mice", Paper presented at the 17th Annual Conference of the West African Society for Pharmacology (WASP), Calabar, April 10-13, 1988. Abstract p30.

(g) H. O. Kwanashie, L. Lege-Oguntoye, T. T. Iyaniwura and K. Gamaniel, "Preliminary studies on the formulation and performance of local laboratory animal feeds", Paper presented at the 17th Annual Conference of the West African Society for Pharmacology (WASP), Calabar, April 10-13, 1988. Abstract p48

ABSTRACT

The effects of prostaglandins in the central nervous system and the uterine smooth muscle were studied.

The prostacyclin analogue, iloprost synchronised the EEG and induced catalepsy in the rat which action might be produced at a site rerrote to but in close functional association with the dopamine receptors, PGE, also induced catalepsy in the mice.

Iloprost exhibited an anticonvulsant action by profoundly decreasing the incidence of seizures and mortality, following strychnine, pentylenetetrazol and maximal electroshock. Iloprost also delayed the onset of tonic seizures following strychnine and pentylenetetrazol.

PGE, and prostacyclin (PGI_2) antagonised strychnine and pentylenetetrazol seizures but were generally less active than iloprost.

The anticonvulsant activity of iloprost, PGE^{1} and PGI_2 might involve a common basic nechanism, possibly involving cyclic AMP and cell membrane interactions,

Iloprost may be a useful tool in the investigation of anticonvulsant mechanisms in the central nervous system.

Dietary mackerel oil supplementation provoked strychnine and pentylenetetrazol-induced seizures in mice which might have resulted from increased production of the 3 series prostaglandins in the cell membranes or from changes in the membrane fluidity, carriei—mediated transport or the activity of certain membrane bound enzymes e.g. Adenylate cyclase.

PGE_1 is a potent compound in stimulating the uterus to contract and calcium plays an important role in this phenomenon.

Low verapamil concentration did not block the contractile actions of PGE_1 in normal conditions but reduced the response to PGE_1 in the presence of high calcium and high potassium.

High verapamil concentration blocked responses to PGE_1 independent of calcium and potassium concentrations.

PGE_1 may not have a major action through potential dependent calcium channels but may act via receptor operated channels as well as being able to mobilise intracellular calcium and its actions thus differ from oxytocin.

PGE_1 produced a significant ($P<0.001$) time dependent increase in cyclic AMP leve.s in the isolated rat uterus.

There appears to be an obvious qualitative difference in the responses elicited by PGE1 and oxytocin on the isolated rat uterus,

From the nature of the contractile responses and the relative absolute velocity ratios ottained for PGE_1 and oxytocin effects, it is possible that cyclic AMP elevation may contribute to the action of PGE_1 and may favour relaxation.

Cyclic AMP may therefore contribute to the apparent difference observed in the coitractile activities of PGE_1 and oxytocin on the isolated rat uterus possibly by enhancing relaxation.

Evidence has been obtained which suggosts that cyclic AMP may not mediate contractile action of prostaglandins but may produce action that favour relaxation.

Piroxicam, a non-steroidal antiinflammatory drug, may antagonise PGE_1 effects on the isolated rat uterus by enhancing relaxation through the cyclic AMP system, probably by inhibiting phosphodiesterase mediated breakdown of cyclic AMP.

CHAPTER ONE

INTRODUCTION

1:1 **Prostaglandins.**

(a) History and Occurrence

The first observation by the New York gynaecologists, Kurzrok and Lieb (1930) while working on female fertility, that human uterus could react by strong contractions or relaxation in reponse to fresh human semen, may be considered to have given birth to what is today known as prostaglandins. The name prostaglandin itself was first proposed by von Euler (1935) in Sweden, who prepared lipid soluble acid extracts of seminal fluid from humans and animals and observed their smooth muscle stimulating and blood pressure lowering properties. He proposed the name prostaglandins because he thought the active principle was coming from the prostate gland, although later studies by Eliasson (1959) showed that the main source was the seminal vesicle

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rather than the prostate gland. It has also been shown earlier that prostaglandin is not a single substance but a family of several chemically related compounds derived from the hypothetical prostanoic acid.

It is now well documented that prostaglandins are very widely distributed in mammalian tissues and body fluids e.g. the kidney, lungs, thymus, spleen, iris, thyroid, adipose tissue, uterus, placenta, gastrointestinal mucosa, central nervous system (CNS), adrenal glands, ovaries, menstrual fluid, amniotic fluid, etc. They have varied and potent biological activities depending on the type of prostaglandin and the tissue or organ involved. The first pure prostaglandins to be isolated were the PGE_1 and $PGF_{1\alpha}$ (Bergstrom and Sjoval, 1960a, b).

(b) Chemistry and Nomenclature

Chemically the prostaglandins are oxygenated cyclic fatty acids consisting of 20 carbon atoms (C_{20}), five of the carbon atoms forming a cyclopentane ring. The fully saturated C_{20} cyclopentane carboxylic acid known as prostanoic

acid is the parent skeleton in the prostaglandin nomenclature. The natural prostaglandins have an α-hydroxyl at C-15. The appended letters A to I refer to the functionality in the cylopentane ring. Thus in the PGE series, the cyclopentane ring has a ketone group at C-9 and an α-hydroxyl group at C-11 while in the PGF series, two hydroxyl groups are present, one at C-9 and the other at C-11 (the subscript α refers to the configuration of the C-9 hydroxyl group). The subscript numerals 1, 2 and 3 refer to the number of double bonds in the side-chains. For all classes of prostaglandins, these bonds appear at the same position and possess the same stereochemistry. A 13-, 14-trans (13E)- double bond is present in all naturally-occurring prostaglandins and thromboxanes (TXs).

For members of the 2 series, the position and stereochemistry of the additional double bond is 5(z). The position and stereochemistry of the third double bond in the 3 series is 17(z). The figure attached (Fig. 1) shows the other classes of prostaglandins as members of the 2 series having double bonds at 5(z) and 13(E). They differ in the ring as follows. PGA_2 has a ketone group at C-9 and a double bond at C-10 whereas the isomeric

PGB and PGC have the alkene unit between C-8, C-12 and C-11, C-12 respectively.

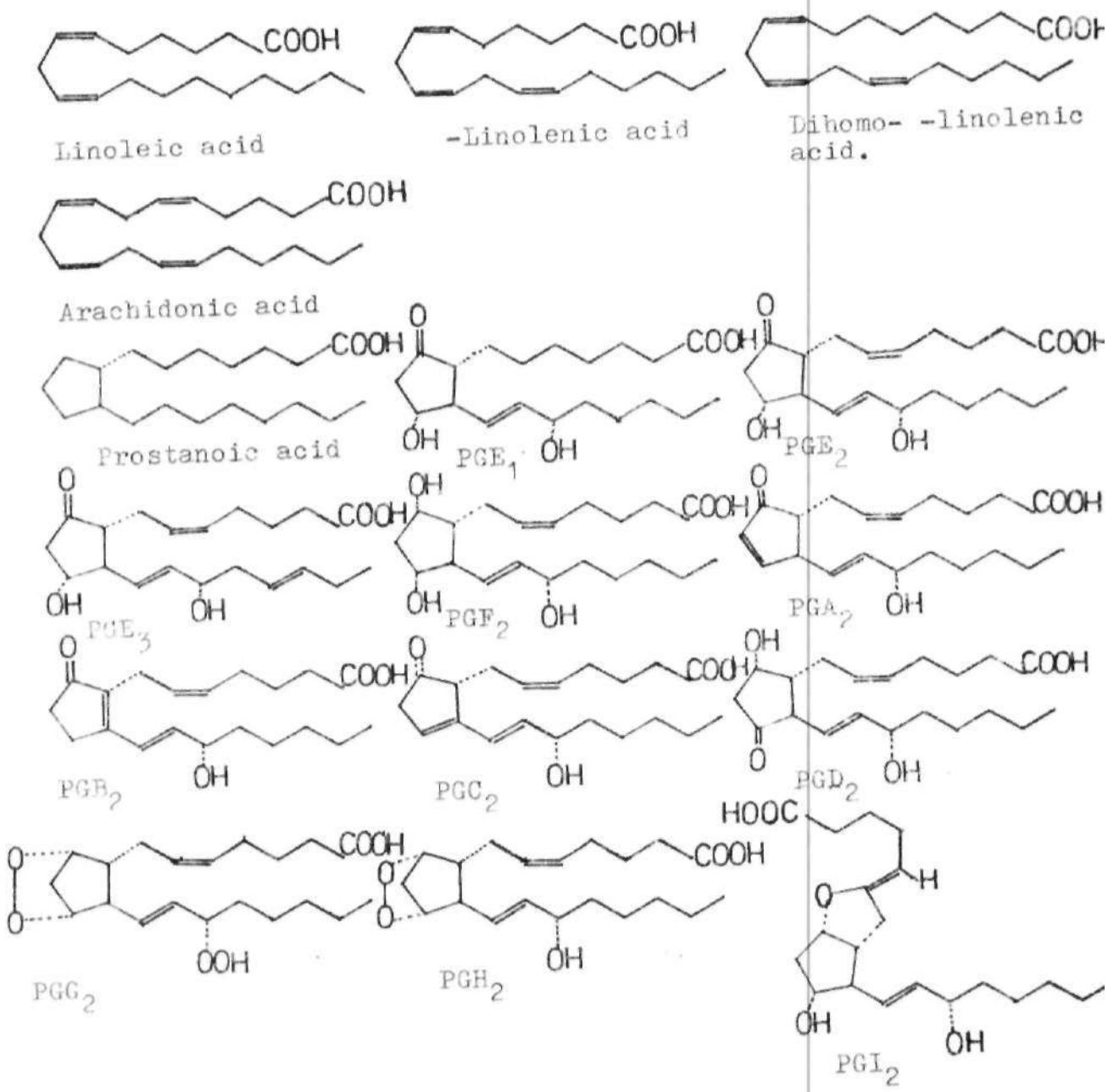


Fig. 1 Prostaglandins: Chemical structure and nomenclature.

PGD_2 is isomeric with PGE_2 and has an α-hydroxyl group at C-9 and a ketone group at C-11. Both PGG_2 and PGH_2 have C-11 linked by an endoperoxide bridge. PGI_2 refers to the furanocyclopentane prostaglandin otherwise known as prostacyclin. For the TXs (eg TXA_2 and TXB_2), the cyclopentane ring is replaced by the tetrahydropyran system. The name TX was given after it was recognised that TXA_2 is produced by platelets and causes their aggregation. However, TXA_2 is very unstable ($t_{1/2}$ =30sec.) and good nmr data has still to be provided for this metabolite.

(c) Source, Biosynthesis and Metabolism

Prostaglandins are fatty acid derivatives formed in most tissues of the body. Since the elucidation of the chemical nature of these compounds, their structural similarity to the essential fatty acids indicated that these dietary fatty acids might be the precursors of prostaglandins in the mammalian tissues and organs. Their most important dietary precursor is linoleic acid (18:2), which is one of the essential fatty acids (Fig. 2). Linoleic acid is ingested in a daily amount of about 10g. A minor

part of this intake is converted in the liver to dihomo-γ-linolenic acid (20:3) and arachidonic acid (20:4; AA). The AA formed in the liver is distributed to most tissues in the body and stored in the cell membranes. In nature the polyunsaturated C_{20} fatty acids are mainly found esterified into phospholipids in the cell membranes. These polyunsaturated fatty acids serve as the immediate precursors in the biosynthesis of prostaglandins of the 1 series (eg PGE_1) and the 2 series (eg PGE_2, $PGF_{2\alpha}$) respectively. Various chemical, physical, neuronal and hormonal factors can stimulate the synthesis of prostaglandins and certain tissue TXs as well (Samuelsson et al, 1975; Vapaatalo and Parantainen, 1978 and Wolfe, 1978). In the first phase of the synthesis, phospholipase A_2 is activated, and this enzyme causes the cleavage of the precursors of prostaglandin synthesis eg AA from the phospholipids of cell membranes. This phase is regarded as the rate-limiting step in prostaglandin synthesis, After liberation into the cytoplasm, AA is immediately attacked by a group of membrane bound enzyme complex generally referred to as "prostaglandin synthetase". The initial step in the transformation of AA to prostaglandins is probably cyclisation of the

carbon atoms C-8 to C-12 and hydroperoxidation at C-11 (elicited by "cyclooxygenase"). This is followed by the introduction of hydroxyl group at C-15 leading to the formation of 2 prostaglandin endoperoxides PGG_2 and PGH_2. The prostaglandin endoperoxides, although possessing biological activity of their own are generally regarded as intermediates in the biosynthesis of prostaglandins. Endoperoxides are transformed to prostaglandins by peroxidase or endoperoxide isomerase. The same endoperoxides are also changed to TXA_2 and PGI_2.

The metabolism of prostaglandins is influenced by a regular dietary supply of linoleic acid, as it has been shown in studies where animals fed with a diet containing small amounts of fatty acids decreased their prostaglandin production. Inactivation of prostaglandins may take place along several routes, giving rise to a large number of metabolites which are finally excreted into urine. The complex excretory pattern of prostaglandin metabolites has hampered the quest for a reliable parameter for prostaglandin turnover. Prostaglandins are rapidly metabolised, with a plasma half life of endogenous prostaglandins being about 1min and that of their main metabolites being 8min. The most important

enzymes participating in prostaglandin metabolism are prostaglandin 15-dehydrogenase (15-PGDH) and 13-reductase as well as 9-ketoreductase.

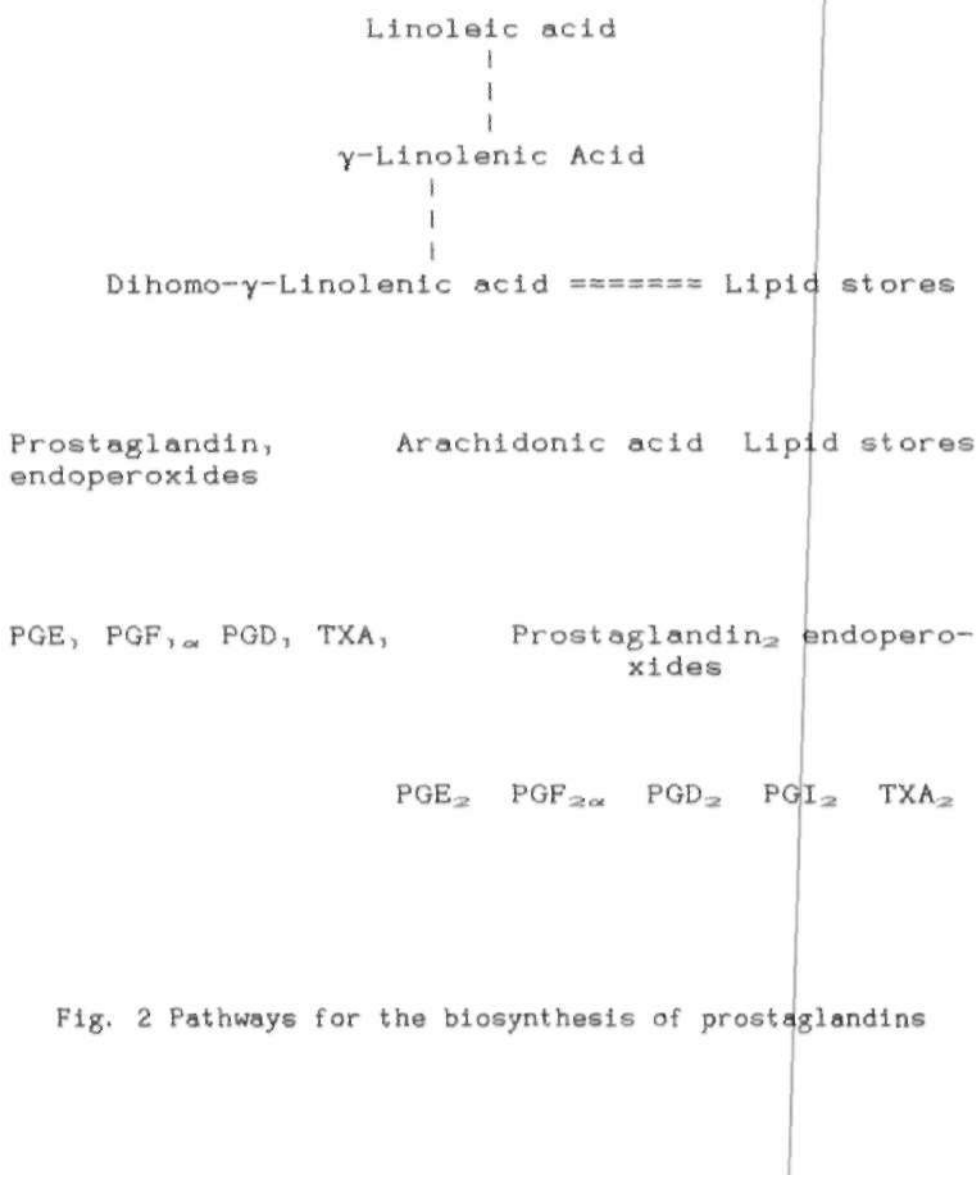


Fig. 2 Pathways for the biosynthesis of prostaglandins

1:2 Prostaglandins and the Central Nervous System

The CNS has a complete system for the biosynthesis of prostaglandins and TXs (Wolfe and Coceani, 1979). There is compelling evidence that prostaglandins are formed at multiple sites in the CNS, both neural and non-neural and interact in a varied manner in physiological and pathological situations. The content of unesterified AA and prostaglandins in the brain and cerebrospinal fluid (CSF) in the physiological basal state is very low (Marion and Wolfe, 1978; Egg *et al*, 1980). Trauma, hypoxia, ischemia, severe hypoglycemia, electroconvulsive shock, or tissue dissection and homogenisation results in a rapid increase in content of free AA and stimulation of prostaglandin synthesis (Bazan, 1976; Foldo et al, 1977; Gardiner et al, 1981).

In contrast to the evidence concerning synthesis of prostaglandins in the brain, little is known about their functional significance in the CNS. The most direct evidence for the central action of prostaglandins derives from iontophoretic studies in which a selective excitatory or inhibitory action was shown when applied in small amounts to brain stem neurons.

The signs of central actions of PGEs frequently described are stupor in the cat following icv injection and sedation in young chicks when given iv. Subcutaneous injection of PGE_1 in mice caused sedation, potentiation of hexobaritone sleeping time and prevention of experimentally induced convulsions (Holmes and Horton, 1968). It has also been observed that icv injection of prostaglandins is able to induce catalepsy (Horton, 1964), potentiate or reduce neuroleptic induced catalepsy (Brus *et al*, 1983), antagonise conditioned avoidance response (Potts et al, 1973) and mouse climbing behavior (Brus *et al*, 1982). These effects were similar to those produced by dopamine receptor antagonists. However, Czlonkowski and Herman (1980), using receptor labelling assays, found that PGE_1, E_2, D_2, or $F_{2\alpha}$ could not displace (^3H)-spiperone from striatal dopamine receptors *in vitro*. Costall and his group (1985) have also proposed that the abilities of prostaglandins to inhibit dyskinetic biting differentially, or to cause asymmetry/circling or to inhibit apomorphine-induced asymmetries (Schwartz *et al*, 1972) may be due to an action at a site beyond the dopamine receptors. It has been postulated that they may be modulators of neurotransmision, thus enhancing or reducing neuronal activity (Wolfe,

1982). Prostaglandins have been shown to act presynaptically and depending on the brain region may either evoke or prevent release of monoamines. They have also been shown to have direct effect on brain stem neurons (Gilmore and Shaik, 1972) which is predominantly excitatory, a direct effect on cerebral regional blood flow (Folco et al, 1977) producing vasoconstriction, an effect on cyclic nucleotides preventing increase in cerebellar cyclic GMP caused by pentylenetetrazol (PTZ) as well as an inhibitory action on picrotoxin- and isoniazid-induced seizures (Rozenkranz and Killam, 1978). However, whether prostaglandins in the brain are associated with the action of particular neurotransmitters is not clear. Dray and Heaulme (1984) using ^{3}H-NA to study the uptake of NA, obtained some evidence which support the concept that in the hypothalamus of the rat, PGE_2 may have a physiological role in controlling release of catecholamines. It has also been shown earlier that sympathetic stimulation leads to increased prostaglandin release in the CNS (Brody and Kadowitz, 1974).

There is evidence which strongly suggests that the presence of pyrogen within the CNS increases the rate at which endogenous prostaglandins are synthesised and released into

the CNS extracellular spaces. The important role of the prostaglandins in thermoregulation was initially reported by Milton and Wendlandt (1971) and Feldberg and Saxena (1971). They suggested that although the central actions of prostaglandins were not confined to the E series, the hyperthermic effects were only produced by PGE_1 and E_2 and that these effects were likely to be due to an action on the preoptic anterior hypothalamus. Similarly the participation of the CNS in the antiarrhythmic effects of PGE_1 and PGE_2 has been demonstrated by Somberg and his coworkers (1977). Mest and Winkler later (1984) presented reports which suggest that prostaglandins PGE_1, $PGF_{2\alpha}$ and PGI_2 may modulate the cardiovascular system through a central mechanism.

Basal levels of prostaglandins and TXs, are generally low in normal brain, but with the onset of seizure activity brain prostaglandin and TXB_2 levels increase dramatically (Steinhauer et al, 1979). The increased AA metabolism measured with the first clinically evident seizure has led to speculation that perhaps some of the products of the AA cascade may serve to modulate convulsive events (Climax and Sewell, 1981) hence a possible significance of these autacoids in this phenomenon. Anticonvulsive effects have been

reported for PGE_1, E_2 and D_2 given systemically or icv (Poddubiuk, 1976; Wolfe, 1982; Forstermann *et al*, 1983). The role of TXs however in the CNS is not clearly understood. Although Wolfe and Pappius (1983) proposed that TXB_2 might activate calcium channels and thus alter synaptic function; later work by McGinley and his colleagues (1985) clearly demonstrated that selectively inhibiting TXB_2 biosynthesis by OKY-1581 (20mg Kg^{-1}) did not contribute to the neuronal events that lead to the seizure episode. There is increasing biochemical evidence that one of the targets of convulsion induced changes is the cell membrane of neurons. Since the production of prostaglandins from free fatty acids is activated after convulsions, it seems that the alteration in the metabolism of lipids in the brain may play an important role in seizures.

1:3 Prostaglandins and smooth/cardiac muscles

Some of the responses believed to be mediated by prostaglandins include contraction/relaxation of cardiac and smooth muscles, modulation of glandular secretions, ion transport, neurotransmitter and anaphylactic

mediator release, potentiation of algesia, maintenance of functional *corpus luteum* as well as control of body temperature.

The commonly accepted indications for prostaglandins are gynaecological and obstetric ones: abortion, induction of labour (PGE_2, $PGF_{2\alpha}$). Prostaglandins have been shown to be important in the uterine vascular response and the implantation process of several species (Kennedy and Zameknik, 1978; Hoffman et al, 1980). *In vivo* studies have revealed that PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ stimulate the uterus to contract at all stages of gestation (Karim et al, 1971; Karim and Sharma, 1971). During menstruation, released prostaglandins favour vasoconstriction which promotes degeneration and desquamation of the endometrium. PGE_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ are probably involved in the expulsive contraction of the myometrium during menstruation and it has been suggested that $PGF_{2\alpha}$ is a primary causative factor in essential dysmenorrhea (Pickles et al, 1965).

All the natural prostaglandins are also formed in the gastrointestinal tract (git). The circular muscle is contracted by $PGF_{2\alpha}$ and relaxed by the PGEs. In the longitudinal muscle, both classes are contractile. When prostaglandins are infused into man or animals, the most typical

reaction is diarrhoea. They also have important functions in secretory processes (Puurunen, 1983) and prevent gastric and duodenal ulcer formation.

Prostaglandins of the E, F, and A series are reported to have positive inotropic and/or chronotropic effects on the heart. The effect of the prostaglandins on the heart muscle differ very much, depending on the prostaglandins and the animals studied (Hodge and Walters, 1975). Prostaglandins are also potent vasoactive agents which are either vasoconstrictors or dilators depending on the animal species, vessels and prostaglandins. PGI_2 and E_1 have platelet antiaggregatory and vasodilatory actions and have been used in some cases of peripheral vascular diseases and also in extracorporeal hemoperfusion.

1:4 Prostaglandins and cyclic AMP/calcium

The observation by Steinberg and his colleagues (1963, 1964) that prostaglandins inhibit cyclic AMP mediated hormone-stimulated lipolysis in isolated fat cells was the first indication of possible link between prostaglandins and cyclic AMP. The regulatory role of prostaglandins on intracellular concentration of cyclic nucleotides at peripheral level as well as in the CNS is well established (Kuehl, 1974; Outila and Vapaatalo, 1984).

Kuo and Greengard (1969) hypothesised that the physiolgical effect of cyclic AMP in various tissues are mediated through regulation (By cyclic nucleotides) of the phosphorylation of specific membrane proteins (Uno *et al*, 1977). These authors further proposed that cyclic nucleotide mediated protein phosphorylation in turn regulated membrane permeability to ions. The cyclic nucleotides are thought to act as second messengers for prostaglandins in many cell types. PGE_1 has been shown to increase cyclic AMP levels in isolated rat heart and to activate phosphorylase (Malik and McGiff, 1976). The effect of prostaglandins have also been suggested to be mediated by enhanced

availability of free calcium to the excitation-contraction coupling process or by the antagonism of the potassium effects (Malik and McGiff, 1976). Metsa-Ketela (1981) found results which do not support the concept that cyclic AMP could mediate the positive inotropic effects of prostaglandins as a second messenger. Rather, he suggested that cyclic AMP increased by PGE_1 or PGI_2 could be responsible for increased relaxation, which might prevent full development of tension. Since prostaglandins may either promote or reduce endogenous cyclic AMP concentrations, it is possible that apart from stimulation of the membrane bound adenylate cyclase enzyme to produce cyclic AMP from the precursor ATP, prostaglandins may also have effects on intracellular phosphodiesterases.

PGE_1 and E_2 (1mg kg^{-1} ; IV) have been observed to lead to 60-70% increase in the cyclic AMP content of the rat brain, whereas $PGF_{2\alpha}$ was much less effective. The stimulation of cyclic AMP was highest in the cerebral cortex and lowest in the cerebellum and brainstem. The cyclic AMP increase in the brain was associated with opposite effects on the behaviour of the animals, e.g. PGEs induced sedation (Stamm and Wellmann, 1973). It has been demonstrated that experimental seizures

alter cyclic nuceotide levels in the brain. Ferrendelli (1983) hypothesised that the elevation of cyclic AMP may be involved in mechanisms inhibiting seizure activity since he found that animals pretreated with cyclic AMP lowering agents (e.g. reserpine, propranolol, aminophylline) showed an enhanced sensitivity of neurons (Gross and Ferrendelli, 1979).

There are several interesting interrelationships between prostaglandins espcially PGE, and calcium. For example modification of the membrane permeability to calcium may account for the antiaggregatory actions of prostaglandins on platelet and on the contractility of cardiac and smooth muscles. The whole group of prostaglandins, their intermediates and precursors are in close functional connection with calcium movement. Calcium is necessary for the activation of phospholipase A_2, the simulatory action of PG-like substances are calcium dependent, and prostaglandins displace calcium from biological membranes. The calcium sensitivity of the contractile proteins may be modulated by the cyclic AMP system. The affinity of myosin light chain kinase (MLCK) for the calcium calmodulin complex is reduced following phosphorylation of MLCK by the cyclic AMP-

dependent protein kinase (Conti and Adelstein, 1980; Ruegg and Paul, 1982). The intracellular receptor protein for calcium in skeletal and cardiac muscles is the troponin-tropomyosin complex, whereas in smooth muscle the receptor protein is calmodulin. Both receptor proteins have been sequenced and have four distinct calcium binding sites, the occupation of which leads to a new conformational state (Cheung, 1980). In troponin C, this change removes another protein troponin I from an inhibitory site on actin which allows the actin-myosin interaction to take place (Potter *et al*, 1977). In the smooth muscle, it is different because the conformational change produced by calcium in calmodulin unveils a hydrophobic site which can bind specific proteins. In this instance calmodulin binds to and activates MLCK which phosphorylates myosin, allowing it to interact with actin (Adelstein and Hathaway, 1979; Ebashi *et al*, 1982). Since cyclic AMP plays a critical role in modulating either secretion or the magnitude of post-synaptic responses, calcium the activator of calmodulin is evidently essential for the neuronal function.

1.5 Objective of the study

Since the discovery of the stimulant activity on uterine smooth muscle by seminal fluid and the subsequent discovery in menstrual and amniotic fluids, prostaglandins have been linked with reproduction. Indeed the commonly accepted use of the prostaglandins are for abortion and induction of labour. *In vivo* studies have revealed that PGE_1, E_2, and $F_{2\alpha}$ stimulate the uterus to contract at all stages of gestation.

Anticonvulsive activities of PGE_1, E_2, and D_2 have also been reported after systemic or intracerebroventricular injection (Poddubiuk, 1976; Wolfe, 1982; Forstermann *et al*, 1983). PGE_1 is also a potent compound in stimulating the uterus and in inhibiting platelet aggregation, mechanisms which are thought to be closely linked with changes in the tissue cyclic AMP concentrations.

The potent and diverse activities of prostaglandins have hampered several attempts that have been made to elucidate their functional roles in both normal body functions as well as in the pathogenesis of some diseases and the mode of action of many drugs.

This study was carried out to explore the

role of prostaglandins in the CNS and the uterine smooth muscle with the aim of studying the events that occur at the cellular and molecular levels during prostaglandins actions. The influence of some prostaglandins on EEG and seizures as well as changes in the cyclic AMP content of the mouse brain during pentylenetetrazole activity and in the presence of prostaglandins will be reported. Furthermore, the effect of tisue enrichment with dietary fatty acid, precursors of prostaglandins, on seizure susceptibility will be presented. In addition, the qualitative difference between the contractile actions of oxytocin and prostaglandins on the isolated rat uterus as well as the roles of cyclic AMP and calcium in this phenomenon will be shown. It is hoped that from critical analysis of these studies, useful correlation may evolve which may provide an insight into the basic mechanism for PG actions and possibly their functional roles in the CNS and uterine smooth muscle.

CHAPTER TWO

MATERIALS AND METHOD

All the study protocols cited below were approved by the ethical committee of the Department of Biomedical Sciences, University of Tampere, Finland.

2.1 The electroencephlogram (EEG)

(a) Implantation of electrocortical and electromyogram (EMG) electrodes in the rats.

The rats were anaesthetised using pentobarbitone (30 mg kg^{-1} i.p.). The rats held firmly with ear plugs in a stereotaxic apparatus were surgically implanted with stainless steel screws (Grass Instruments). The sites of implantation include the frontal cortex (FC), optical cortex (OC), and midbrain reticular formation (RF). The stereotaxic coordinates for the sites were as follow:-

FC:	anterior-posterior	+1.9mm
	lateral	+1.5mm
OC:	anterior-posterior	+9.2mm
	lateral	-2.8mm
RF:	anterior-posterior	-3.8mm
	laterior	+2.0mm
	vertical	-8.2mm

The implanted electrodes were connected to the male connector as follows:-

Monopolar electrodes	from	upper surface of mouth	:	No. 1
"	"	frontal cortex	:	No. 2
"	"	optical cortex	:	No. 3
Bipolar electrodes	"	brainstem RF	:	No. 4
Two EMG electrodes	"	the neck	:	No. 5 & 6

The surface location of these brain areas was first marked before drilling the skull and implantation of the electrodes. The implanted eletrodes were held firmly in place using dental cement. The neck muscle was similarly implanted for EMG recording. The EMG electrodes were made of stainless steel insect pins No. 00 which were bent at the tip into hooks and inserted proximally to the head into the semispinalis capitis muscle on the left side of the dorso-lateral aspect of the

rat. The rats implanted were allowed 24hr with an angle poised lamp for full recovery from anaesthesia before use.

(b) Preparation of the 7P511H EEG Amplifier for recording.

Before using the EEG machine, it was calibrated as follow: -

paper tracking checked and chart speed selected. The control settings were selected as indicated below:

CAL-USE 50uV
1/2 AMP low frequency 1Hz
uV/mm-mV/cm .....uV/mm
Sensitivity 5
Equaliser ....adjust so that 50uV gives 1cm
Baseline ....rotate untill pen is at mechanical zero.
1/2 AMP Hi freq (J6) 30KHz
1/2AMP Hi freq (pens) 90Hz
Both 60Hz filters .....out
power .....on

With MASTER WRITE switch at CHART and PEN, CAL G1 negative push button was depressed and held until pens returned to baseline (this gave an upward movement). CAL G1 negative push button was released (producing downward movement). The deflection was measured by metric rule. If the deflection was not 1cm exactly, the equaliser control was adjusted clockwise for higher response or anticlockwise for smaller response (Adapted from model 79 EEG/polygraph instrument manual 1974). The instrument is then ready for recording.

(c) EEG recording in the rat

The electrodes of the implanted rat were attached to a male connector and hooked into the female connector. The sequence of the implanted electrodes to the male connector were as mentioned in (a) above. The female connector was positioned in a screened cage (to avoid electrical interference). The rat was allowed 1hr to settle down before the commencement of EEG recording. Prior to the recording, the rat was injected with the solvent vehicle and a control reading taken for 30min after which the test drugs were injected and the EEG recording continued for 3hr. The chart

speed during normal recording was 2.5mm/sec but at appropriate times the speed was changed to 25mm/sec to facilitate analysis of voltage and peak frequency. Fast chart speed was used at 5, 10, 15, 30min after vehicle administration and 5, 10, 15, 30, 60, 90, 120 and 180min after drug administration. At each period the fast chart speed was allowed to run for 12sec only. At the end of each recording, the calibration response was again checked to ensure optimal response from the instrument during the recording.

2.2 Cataleptic behavioural effects of iloprost and PGE_1 on the rats

Catalepsy was measured using the method of Costa*let al.* (1978). Adult male rats were kept in a quiet room with an ambient temperature of 24±2°C. The rats were allowed free access to food and water before every study commenced. The rats were placed in individual perpex cages equipped with a horizontal bar fitted 6cm high. Thirty minutes were allowed before drug treatment, for the rats to adapt to the new environment. Control rats, without drugs were observed concurrently with the test rats injected with either pimozide,

iloprost, PGE, or amphetamine. All the observations were made for a period of 2 hours.

In testing for catalepsy, the animals were held gently by the tail and the front limbs gently placed on the bar, while the hindlimbs were left on the floor of the cage. This position was maintained before the supporting hand was slowly and cautiously withdrawn.

Pimozide (Janssen Pharmaceutical, Brussels, Belgium) was prepared by suspending in Tween 80 (3% w/v) and diluting further with normal saline. From a stock solution of PGE, (UpJohn, Kalamazoo) in ethanol, appropriate dilutions were made using normal saline. Similarly, iloprost (Shering, AG Berlin) was diluted with normal saline. Amphetamine (Smith, Kline & French Lab) was dissolved in normal saline. All the drugs were prepared freshly and were administered i.p. except for amphetamine which was administered s.c. The rats were placed on the horizontal bar at 10min. interval to determine the onset of catalepsy. Readings were taken 2 h post-drug administration. The incidence of catalepsy as well as the intensities were measured in minutes and seconds, and the values were scored as follows:

0 - 5 sec. = score 0;

6sec.-1min. = score 1;

1min. 1sec.- 5min. = score 2;

5min. 1sec. - 10min. = score 3;

10min. 1sec. - 20min. = score 4;

20min. 1sec. - 30min. = score 5.

Cataleptic readings for pimozide (5mg kg^{-1}), PGE, (0.1mg kg^{-1}) and iloprost (0.05mg kg^{-1}) were obtained. The inhibitory effects of amphetamine (2mg kg^{-1}) on pimozide, iloprost and PGE, - induced catalepsy were determined by administering the latter drug 5 min. before amphetamine.

2.3 Convulsive Seizures

(a) Effects of iloprost, PGE, & PGI_2 on chemically and electrically induced seizures

Adult NMRI mice (20 - 30g) were used as experimental animals. Adult male mice were preferred to avoid periodic endocrine changes on brain excitability (Wooley et al, 1961). The animals were kept singly in cages and provided with food and water *ad libitum* before the

commencement of the experiment. The experiments were performed in a quiet temperature-controlled room (22±1°C). After each experiment, the mice were observed for at least one hour so as to record any death resulting from the injection of strychnine (STR) and leptazol (pentylenetetrazol, PTZ) or maximal electroshock (MES) convulsions. The experiments were carried out during the same period of the day using the prostaglandins in a randomised fashion.

In the mouse the most powerful convulsive state is characterised by tonic extension of the hindlimbs and a backward thrust of the head and forelimbs. In this study the time from the beginning of injection of the chemical convulsants to the stage of persistent convulsion leading to tonic hindlimb extension was determined. Lack of tonic extension of the hindlimbs and a backward thrust of the head and forelimbs was regarded as no convulsion for both electroshock and chemical convulsion.

Three convulsants (PTZ, STR, and MES) were used. PTZ and STR were dissolved in 0.9% NaCl solution. Iloprost (ZK36 374) solution was further diluted to the appropriate concentrations using saline while PGE_1 and PGI_2 were dissolved in absolute ethanol and further diluted with saline.

All the drugs were freshly prepared on the days of the experiments. The pretreatment times for iloprost, PGE_1 and PGI_2 (10min) before injecting the chemical convulsants or subjection to MES were established in a preliminary study. The control mice injected with PTZ (108mg kg^{-1}), STR (1.25mg kg^{-1}) or subjected to MES (194v, 150pps, 200msec transcorneally) were studied concurrently with the test mice. The chemical convulsants were administered subcutaneously (sc), while the prostaglandins were administered intraperitoneally (ip), in a volume of 0.01ml kg^{-1} body weight of the mouse, as these routes were found to produce consistent results. Each mouse was used only once for the seizure experiment, after which it was killed by carbon dioxide. Routine controls were used to ensure that the results were based on *threshold responses*.

(b) Dietary supplementation of mackerel oil on seizure susceptibility in mice.

Three groups of male NMRI mice were used in this study. One control group (n=50) was treated with a standard feed (mouse cubes). A second and third groups were treated with the standard feed

in which 10% and 20% W/W mackerel oil were incorporated respectively. Both standard and mackerel oil supplemented feed were purchased and prepared at the Animal House, Department of Biomedical Sciences, University of Tampere, Finland. The feeding program was for 10 days during which the weight gain of the mice in both control and test groups were monitored. All the groups were tested against strychnine and leptazol seizures, in the manner described in 2:2 above, at the end of the feeding program.

The results were all analysed statistically using the chi-squared test with Yates correction for continuity (for incidence of tonic seizures) and Students t-test (for onset of seizures).

2.4 Cyclic AMP Determination in The Brain Tissue

(a) Tissue fixation and sample preparation

Because the prostaglandins used in our experiment exhibited a consistent and significant anticonvulsant effects against PTZ-induced seizures in mice, this convulsant was selected for the determination of the changes in cyclic AMP levels during convulsive and anticonvulsant actions of PTZ and prostaglandins repectively. Mouse brain samples were taken from saline control and PTZ-treated control groups (15min post injection) as well as from prostaglandin pretreated saline and PTZ test groups. The brain tissues were rapidly fixed by exposing the mice to a high intensity focussed microwave irradiation which allowed tissue fixation to occur in less than 250mSec (Palmer, 1983) and enabled brain dissection to be carried out at room temperature. This method also reduced the activity of brain cyclic nucleotides to zero. The brain samples were stored at -20°C until reconstituted for assay by radioimmunoassy (RIA)

Whole brain tissue samples were homogenised with MSE (polytron) homogenizer for 30sec, in 1ml

of 5% trichloroacetic acid (TCA) solution which contained 0.05N HCl. The percentage recovery by these methods were determined by adding tracer amounts (50µl; =15000cpm) of ^{3}H-cyclic AMP to the original tissue homogenate. The samples were then centrifuged (ultracentrifugation) for 15min at 5000xg (RC-5). The precipitate was kept for protein determination (see procedure below). The supernatant was washed four times with 1.5ml of water saturated ether (Albano et al, 1974) centrifuging at each interval for 15min at 5000 rpm to remove the TCA. The washed supernatant was evaporated to dryness in an air stream at +50°C overnight. The residue was then dissolved with 1.0ml of 0.5N HCl and cyclic nucleotides were separated with anion exchange (Dowex-50) columns. The columns were first washed with 0.1N HCl before the samples were applied. Distilled water was used to elute the samples through the columns in the following manner:-

1. First 0.5ml of water was discarded
2. The next 1.0ml contained cyclic GMP
3. The next 0.5ml was also discarded
4. Next 2.5ml contained cyclic AMP

Both cyclic AMP and cyclic GMP were collected and evaporated to dryness. cyclic AMP was reconstituted in 500µl of 50mM Acetate buffer(pH

4.0) for assay by RIA. To evaluate the recovery, 100µl of the resuspended sample was transferred into a scintillation vial and 2.5ml scintillation liquid (Aqualuma, Lumac AG Basel Switzerland) was added.

(b) Cyclic AMP preparation and counting

The Amersham (Radiochemical Centre England) cyclic AMP assay protocol (1979) was adopted in this study. Cyclic AMP determination was performed by the protein binding assay of Gilman (1970). The basic principle of RIA is the competition between radioactive (Ag*) and nonradioactive antigen (Ag) for a fixed number of antibody binding sites as shown schematically below: -

Labelled antigen + specific antibody = labelled antigen anti-
Ag* Ab body complex (Ag*Ab)

+

unlabelled antigen (Ag)

↓

unlabelled antigen antibody complex (AgAb)

So, if increasing amounts of non-radioactive antigen (standard or unknowns) and a fixed amount of radioactive antigen are allowed to

react with a constant amount of antibody, a decreasing amount of radioactive antigen is bound to the antibody. This relationship can be expressed as a standard curve and the amount of unlabelled antigen in a sample determined by interpolation from this curve. Separation of bound from unbound antigens can be achieved by a number of ways eg. by precipitation. The step by step procedure adopted for the assay is outlined below: -

i. 14 assay tubes plus additional tubes for unknowns were placed in duplicates into a rack which was kept in an ice/water bath (±0°C) throughout the assay. The tubes were labelled & arranged according to the protocol shown in Table 1.

ii. 50µl of tritiated cyclic AMP was pipetted into each tube.

iii. 50µl of 50mM acetate buffer (pH 4.0) was pipetted into tubes 1 & 2. These tubes were for the determination of 0 dose binding (ie. binding in the absence of unlabelled cyclic AMP).

iv. 50µl of each dilution of the standard cyclic AMP (diluted to increasing strengths of 0.5, 1.0, 2.0, 5.0 and 10.0 pmol/assay tube) were added to each successive pair of assay tubes (tubes 3 - 12 following the protocol shown in Table 1 below).

Table 1. Cyclic AMP assay protocol.

Tube No.	(H^3)cAMP	Buffer	Standard	Unknown	Blank	Antiserum	Remark
1,2	50µl	50µl	-	-	-	100µl	0 dose
3,4	50µl	-	50µl	-	-	100µl	0.5 pMol
5,6	50µl	-	50µl	-	-	100µl	1.0 pMol
7,8	50µl	-	50µl	-	-	100µl	2.0 pMol
9,10	50µl	-	50µl	-	-	100µl	5.0 pMol
11,12	50µl	-	50µl	-	-	100µl	10.0 pMol
13,14	50µl	-	Blank	-	100µl	-	Blank
15 etc	50µl	-	-	100µl	-	100µl	Unknown

v. 50µl of each unknown (brain cyclic AMP extracts) were added to the appropriate assay tubes in duplicate

vi. 100µl of protein kinase (antiserum) was added to each assay tube except 13 and 14 which were used to determine the assay blank (nonspecific binding). The tubes were capped and vortex mixed for a few seconds.

vii. The ice bath containing the tubes was placed in a cold room (2 - 4°C) for one and a half hours.

viii. 2.5ml of ice cold $(NH_4)_2SO_4$ solution was added to as many tubes as the centrifuge could carry in one batch. The tubes were votex-mixed for a few seconds then replaced in the ice bath and allowed to stand for 5min (timed from the addition of $(NH_4)_2SO_4$ to the last tubes.

ix. The tubes were centrifuged (15min, 5000 g) five minutes after the addition of $(NH_4)_2SO_4$.

x. The tubes were removed from the centrifuge and the supernatant liquid decanted. The tubes were placed upside down on a tissue to drain (15min).

xi. A piece of tissue was used to carefully wipe out excess liquid from the neck of each tube without disturbing the precipitate.

xii. All the tubes were processed in the same

way.

xiii. 100µl of 0.05N NaOH was used to dissolve the precipitate followed by heating on blocks (+ 56°C) for 15min.

xiv. 2.5ml Scintillation liquid (Aqualuma® Lumac AG, Basel, Switzerland) was added, vortex-mixed and taken to the liquid scintillation counter for measurement of radioactivity.

(c) Protein determination

The protein content of the sample was measured by the method of Lowry et al (1951). The pellet of TCA extract was dissolved in 1ml of 1mM NaOH. Reagent A (20% Na_2CO_3 in 1N NaOH) was diluted 1:10 water. The diluted reagent A was then used to prepare reagent B in the following manner: 100ml of the diluted reagent A was mixed with 1.0ml of Na-K-tartarate + 1.0ml $CuSO_4$. 2.0ml of the resultant reagent B was mixed with 1ml of the protein sample (vortex-mixing). 10min later, 100ul of Folin-Ciocalteau was added and mixed together. After 30min the processed sample was taken to the spectrophotometer for measurement of absorbance at 578nM wavelength. The photometer was first zeroed with sample before measurement commenced.

(d) Calculations

The average of each set of duplicate counts (counts per minute, cpm) was first taken. The average net counts for all the samples were calculated by subtracting the average net counts of the blank counts per minute. The concentration of cyclic AMP in each sample tube was determined and expressed in picomoles (pmol) per mg protein. The sample with concentrations above the range of the standard curve was diluted and reassayed. The values obtained were then multiplied with the appropriate dilution factor.

2.5 Effects of PGE_1 and oxytocin on the isolated rat uterus

Female Wistar rats (240-260g) locally bred in the Animal House, Department of Biomedical Sciences, University of Tampere, Finland, were used. The rats were anaesthetised by urethane (100mg Kg^{-1} ip). The uterine horns were removed and mounted on 10 ml isolated tissue bath in De Jalon solution (composition in mM: NaCl 154; KCl 5.63; $CaCl_2$ 0.41; D-glucose 2.52; $NaHCO_3$ 5.95) at a temperature of +37°C and gassed with O_2. The uterine horns were connected to isotonic force

transducer (Model 386, Harvard Apparatus, Millis, U.S.A.) and a recorder (Biograph 2120, Harvard Apparatus, Millis, U.S.A.). The contractile signals were passed through a waveform analyser (Data 6000, Data precision, Danvers, U.S.A.) for further analysis. PGE_1 (Upjohn Co., Kalamazoo, Mich., U.S.A.) was dissolved in absolute ethanol; Oxytocin (Star Pharmaceutical Co. Tampere, Finland) was dissolved in distilled water, while piroxicam (Star Pharmaceutical Co. Tampere, Finland) was dissolved in 0.1N NaOH. Further dilutions were made in De Jalon solution. All the drugs were freshly prepared and the solvent vehicles were tested separately and found to have no effects on their own in the appropriate dilutions. The effects of PGE_1 and oxytocin were tested separately and analysed using one minute contact time between the drugs and the tissue. For interaction studies between PGE_1, isobutylmethylxanthine (IBMX) and piroxicam, IBMX ($1 \times 10^{-6} - 5 \times 10^{-5}$M; Aldrich Chemie, GmbH, Steinheim, FRG) or piroxicam ($1 \times 10^{-7} - 1 \times 10^{-3}$M) were administered into the tissue bath, 5min prior to the administration of PGE_1.

2.6 Effects of PGE_1 on the isolated rat uterus in the presence of verapamil

Adult Wistar rats inbred in the Animal House weighing between 180 - 200g were treated with stilboesterol (1mg kg^{-1} sc) 48hr prior to the experimemt. The uterine horns were surgically removed after anaesthetising the rats with urethane (1000g kg^{-1}ip) and mounted in isolated tissue bath containing either normal De Jalon solution, high potassium (100mMol K^+) or high calcium (4.87mmol Ca^{++}) De Jalon solutions at a temperature of 37°C and gassed with O_2. The uterine horns were connected to isotonic force transducer of Ugo Basile recording microdynamometer (No. 7050 Research Apparatus Italy). The equilibration period was 15 - 20 min. PGE_1 solution was prepared as in 2.2 above and normal dose response curves were obtained for PGE_1 separately in the different De Jalon solutions prepared. Log dose response curves were then plotted from the values obtained.

2.7 Cyclic AMP assays from the uterine smooth muscle

The uterine tissues were set up in isolated tissue baths in the same manner as in 2:4 above. Four preparations were stabilised at the same time for 45min, after which a drug was given in a 200µl volume. The uterine tissue samples were quickly removed at different times (5, 10, 20, 30, 40, 50, and 60 sec) post PGE, administration and were frozen by Wollenberger clamps precooled in liquid nitrogen. The fixation procedure from cutting of suture to frozen tissue took 1-2sec. Control preparations without drugs were also assayed concurrently in a similar fashion. The uterine muscle preparations were then stored for the analysis of cyclic AMP at -20°C.

The tissues were pulverised by a microdismembrator (B. Braun, Melsungen, Germany) in a teflon vial precooled in liquid nitrogen with a 13 g steel ball. The pulverised uterine tissue was put in polyethylene vials (Eppendorff GmbH, Eppendorff, Germany). Samples were then suspended in 0.4M $HClO_4$ (perchloric acid) and homogenised using MSE-microwave homogeniser. Recovery isotopes were added to the tissue homogenates and centrifuged in Eppendorff centrifuge at 12000 g for 15min. The

supernatants were neutralised with 1.44N Na_2CO_3 (to precipitate $HClO_4$) while the pellets were kept for protein determination. The neutralised sample was further centrifuged at 12000 g for 15min then the supernatant was taken and evaporated to dryness in an airstream +50°C overnight. The residues were dissolved in 50mM acetate buffer pH 4.0. Cyclic AMP determination and counting were performed by the method of Gilman (1970), as has been described above. The pellets of the perchloric acid were dissolved in 1ml of 1M NaOH and used for the measurement of protein content (Lowry et al, 1951). To evaluate the recovery, 100µl of the resuspended sample was transferred into a scintillation vial and 2.5ml scintillation liquid was added. From the radioactivity, the recovery of the extraction was calculated and taken into consideration in the final concentrations.

CHAPTER THREE

RESULTS

3.1 *EEG effects of iloprost in the rat*

Iloprost synchronised the EEG (FC = frontal cortex; OC = optic cortex; RF = brainstem reticular formation) but apparently increased electromyogram (EMG) activity in the rat (Fig. 3b).

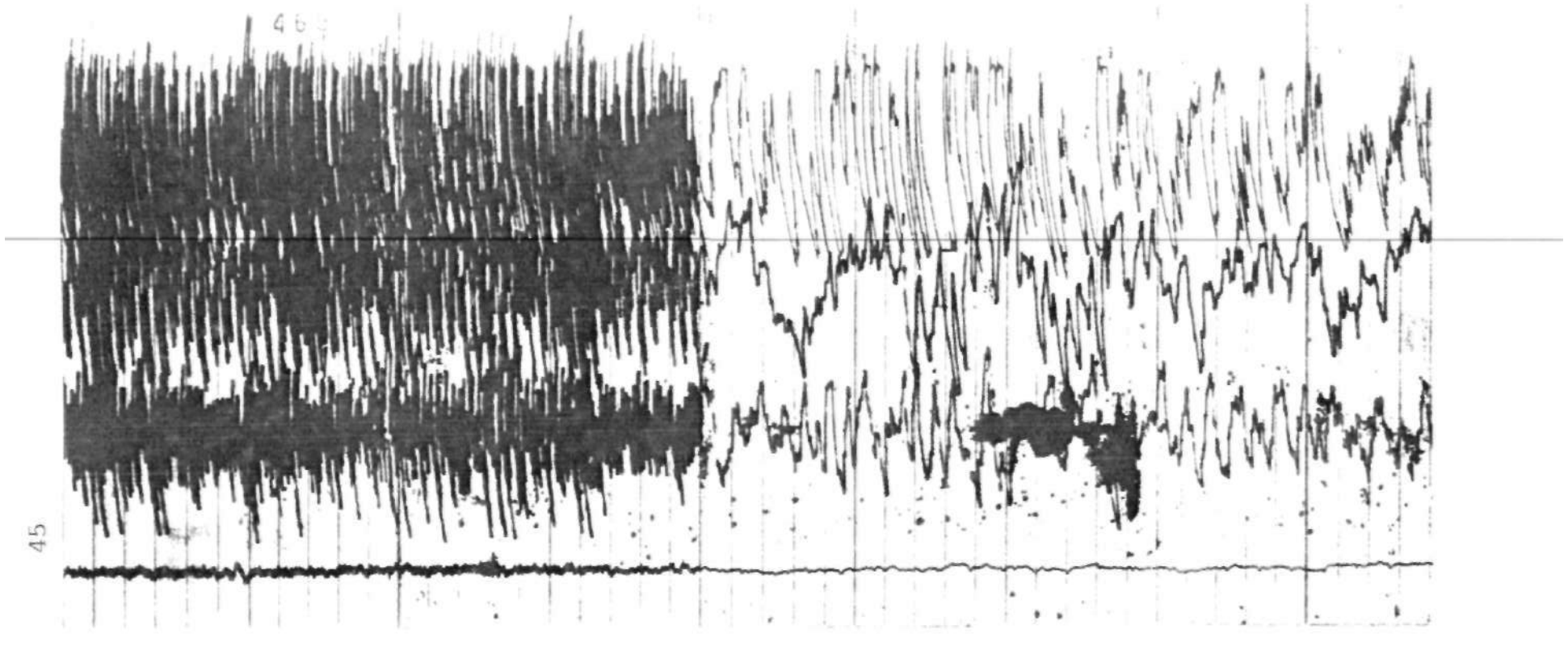


Fig. 3a Effect of normal saline on EEG (FC = frontal cortex; OC = optical cortex; RF = pontine reticular formation) and EMG in the rat.

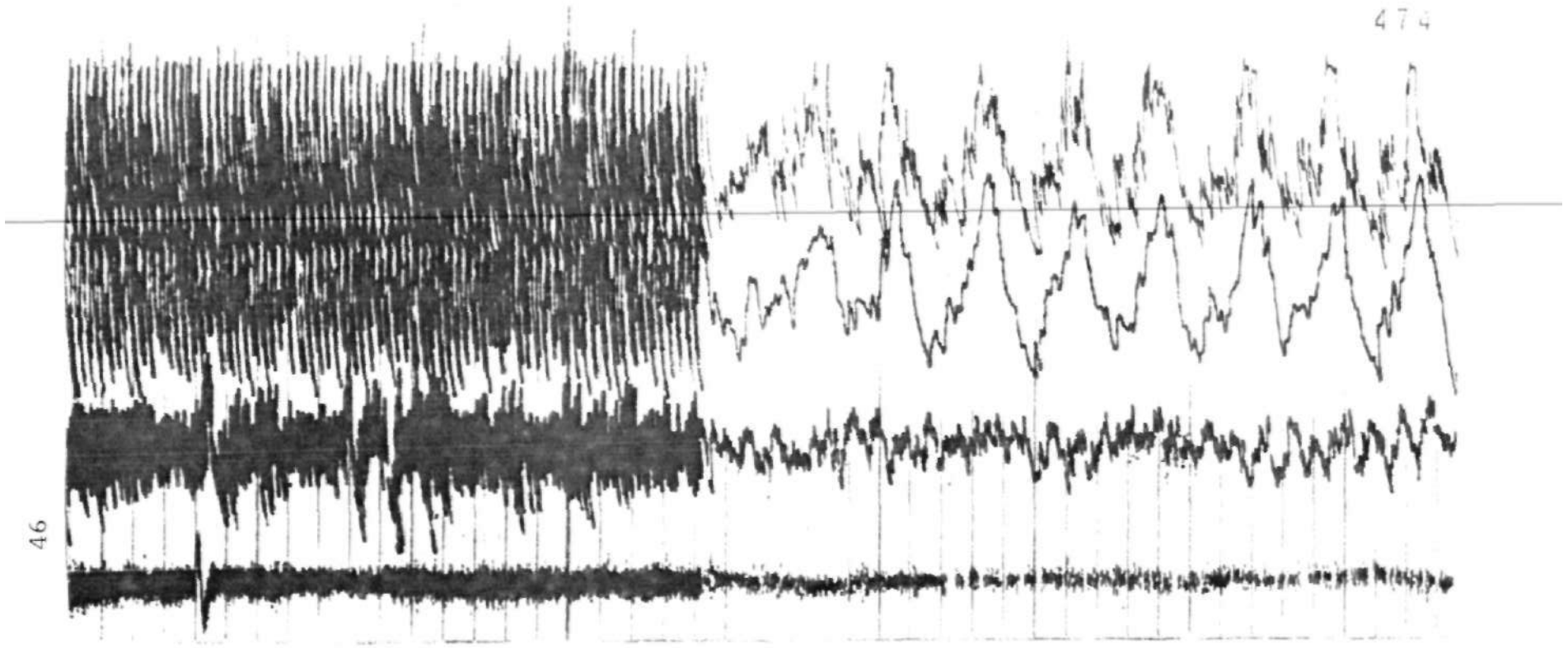


Fig. 3b Effect of iloprost (0.05mg/kg i.p.)on the EEG (FC - frontal cortex; OC - optical cortex; RF - pontine reticular formation) and EMG in the rat.

3.2 *Cataleptic behavioural effects of iloprost and PGE, on the rat*

Pimozide (5mg kg^{-1}) induced a time-dependent catalepsy in the rat (Fig. 4a). The onset of catalepsy was within 10min. Peak effects were recorded at the end of 2hr. Iloprost (0.05 µg kg^{-1}) also induced a profound catalepsy in the rat with a quick onset and the peak effect was observed within 20min. (Fig. 4b). The intensity of catalepsy was high when compared with that of pimozide and PGE_1. There was a gradual decrease in the mean cataleptic scores after the peak effects at 20 and 30min. which later stabilised between 1.29 and 1.41.

Like pimozide, PGE_1 (0.1 mg kg^{-1}) induced catalepsy time dependently (Fig. 4c). The onset of catalepsy was within 10min, but the peak effect was observed at 60min. The intensity of catalepsy within the first 30min. was higher than that of pimozide. Cataleptic scores increased gradually with time until the peak effect at 60min. when a gradual decrease also ensued until the end of 2hr.

Amphetamine (2mg kg^{-1}) antagonised pimozide and iloprost induced catalepsy. Although amphetamine completely abolished iloprost induced catalepsy, some trace of catalepy could still be observed for pimozide 100min. after the two drugs were administered.

Fig. 4a Pimozide induced catalepsy in the rat
Mean cataleptic scores measured at 10min interval for 2h.

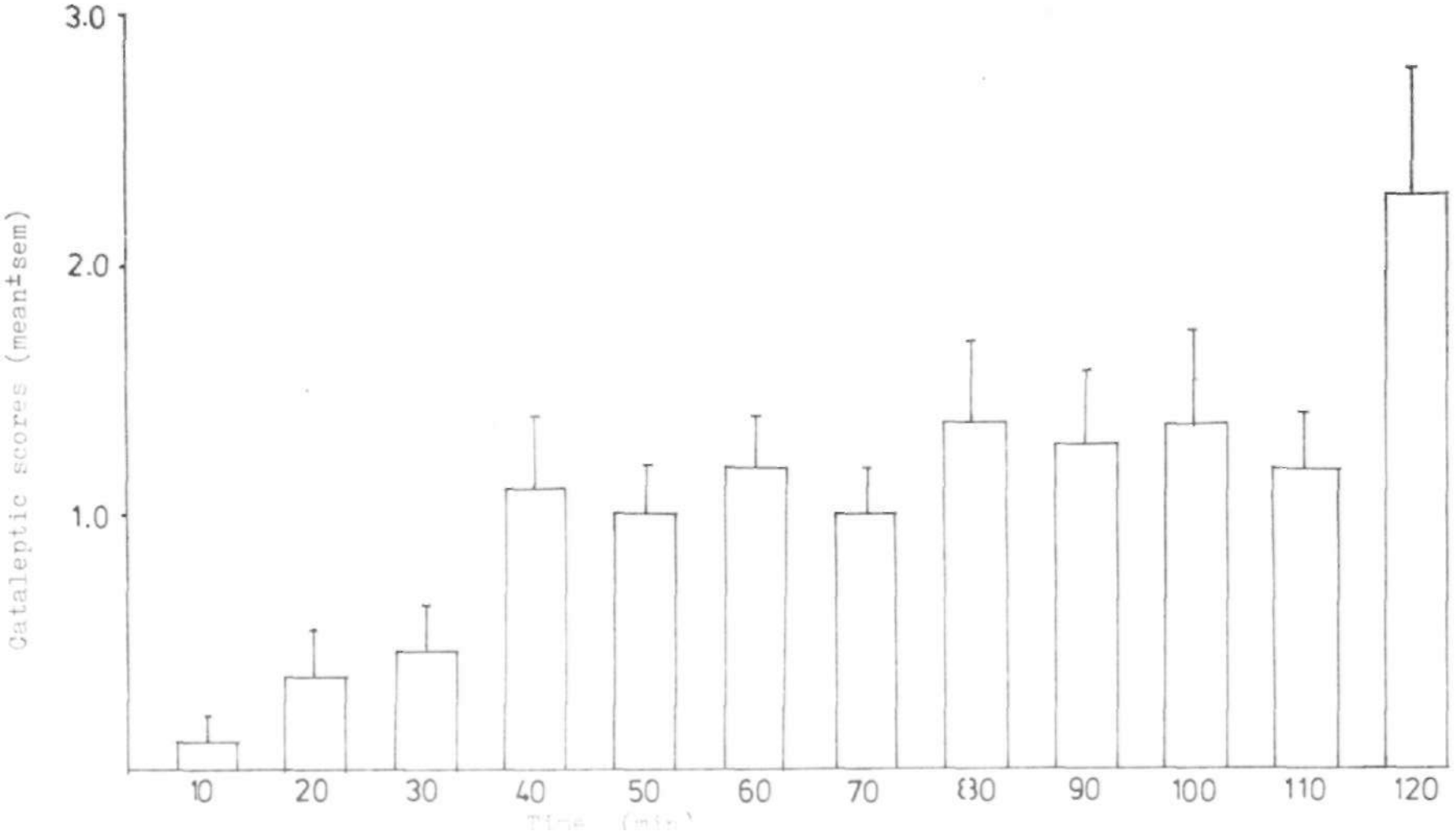


Fig. 4b Iloprost (0.05mg/kg i.p.) induced catalepsy in the rat. Mean scores measured at 10min interval for 2h.

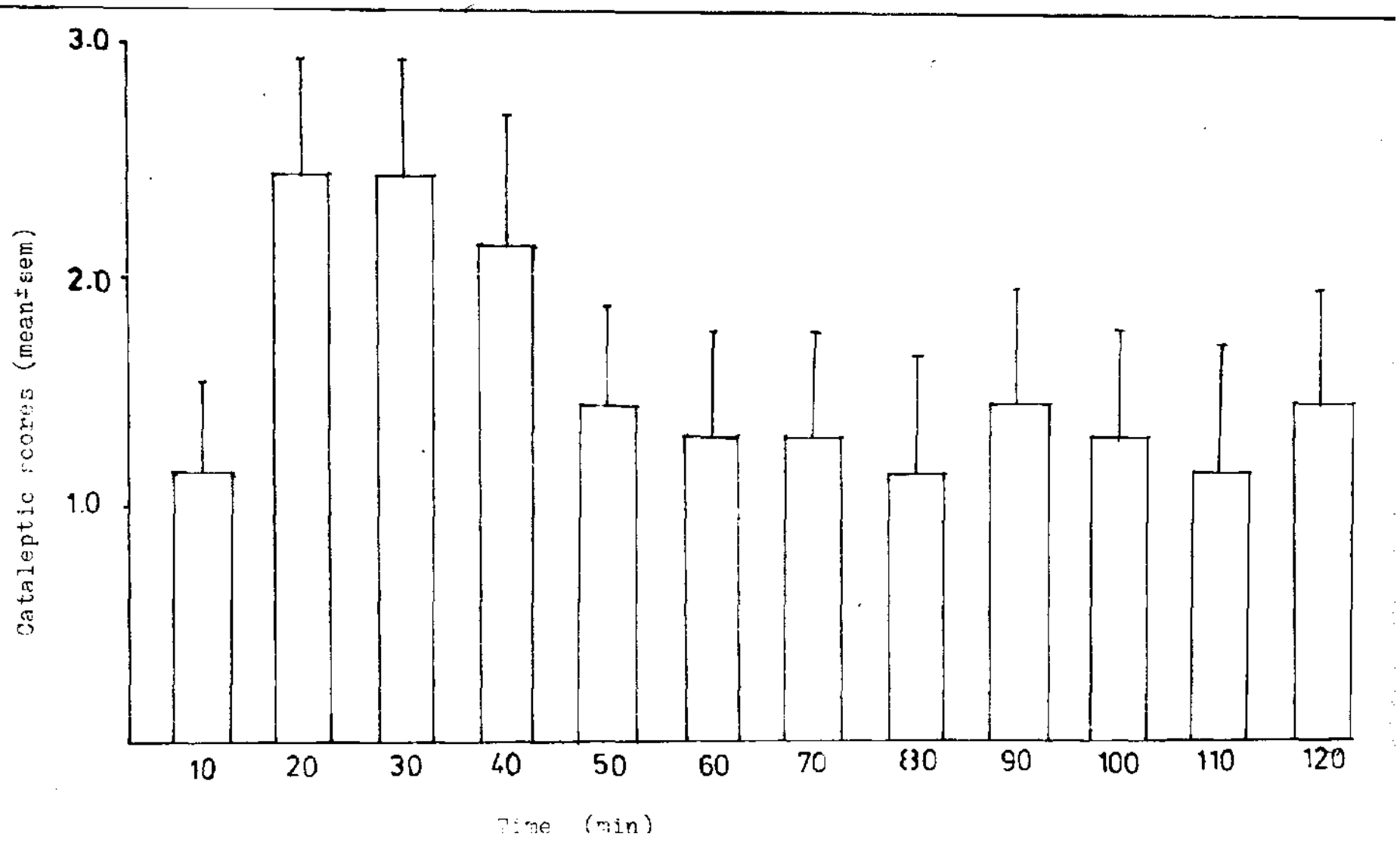
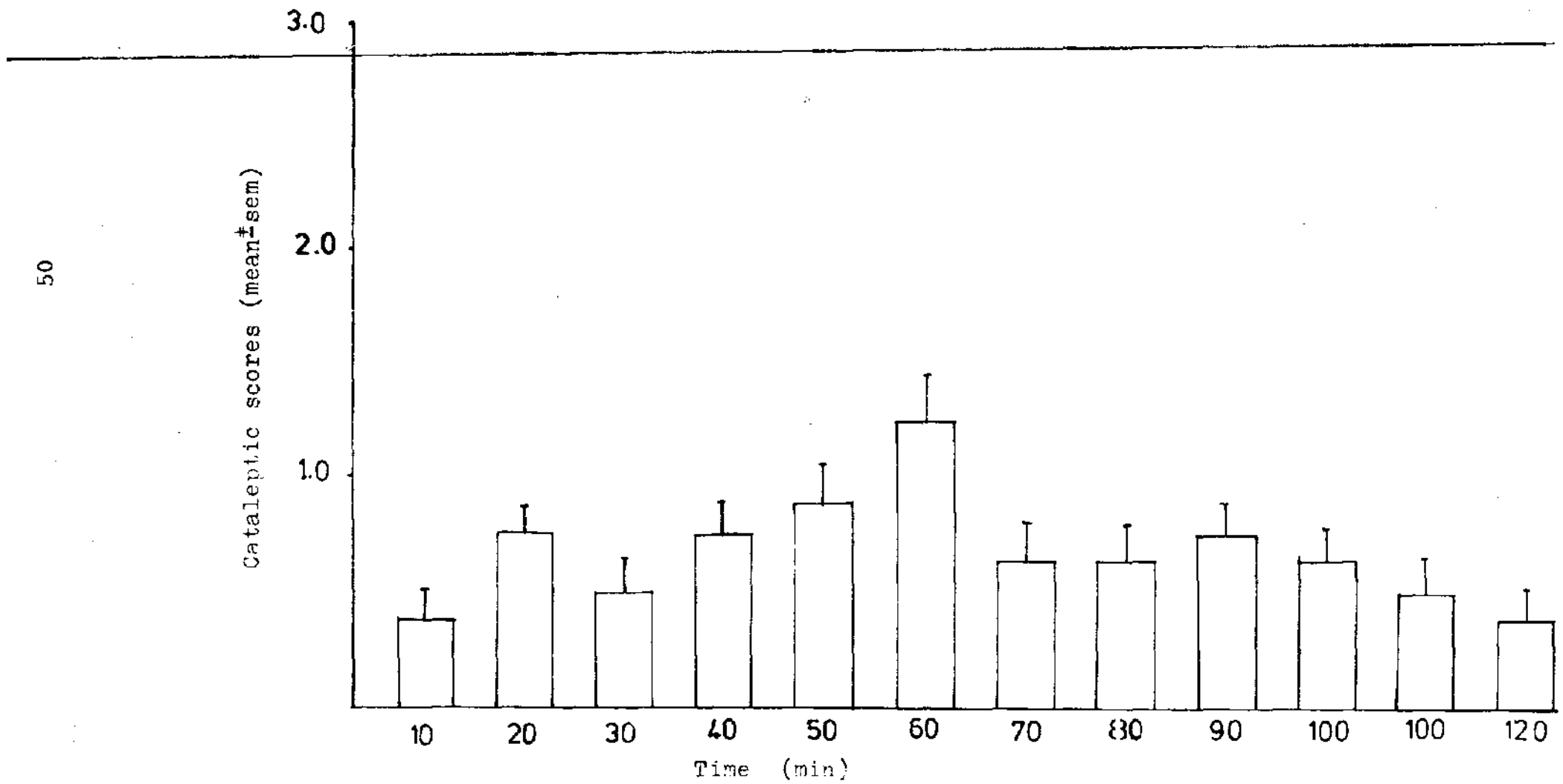


Fig. 4c
PGE (0.1mg/kg i.p.) induced catalepsy in the rat. Mean scores measured at 10min interval for 2h.



3.3 *Effect of Iloprost, PGE, and PGI_2 on chemically and electrically-induced seizures in mice*

Iloprost

Iloprost (8.0-63.0 μg kg^{-1}) exhibited a potent dose-related anticonvulsant effect against STR (Table 3.3) PTZ (Table 3.4) and MES (Table 3.5). High doses (32.0-63.0 μg kg^{-1}) of iloprost abolished completely the STR seizures and mortality, while lower doses reduced profoundly the incidence of tonic hindlimb extension and delayed significantly the onset of STR convulsions ($P<0.05$). Similarly, iloprost markedly reduced the incidence of seizures and mortality following PTZ. At the highest dose tested, iloprost (63.0μg kg^{-1}) also delayed the onset of these seizures significantly ($P<0.05$). In another experiment, iloprost (32.2 μg kg^{-1}) was able to reduce significantly the incidence of tonic hindlimb extension following MES. At large doses (32.0-63.0 μg kg^{-1}), iloprost apparently reduced the incidence of tonic seizures and abolished the mortality induced by MES.

PGE$_1$

High doses of PGE$_1$ (100-200 μg kg^{-1}) reduced markedly the incidence of tonic seizures induced by STR (Table 3.3), and apparently delayed the onset. The mortality following this type of seizures was reduced, compared to the controls. PGE$_1$ (100 μg kg^{-1}) also reduced the incidence of PTZ (Table 3.4) seizures significantly ($P<0.05$). PGE$_1$ (50-200μg kg^{-1}) profoundly delayed the onset of PTZ seizures and reduced the mortality. PGE$_1$ produced a dose-dependent effect against MES (Table 3.5). Low doses of PGE$_1$ (25-50μg kg^{-1}) seemed to reduce the incidence of seizures but increased the mortality. On the other hand, higher doses of PGE$_1$ (100-200μg kg^{-1}) reduced the mortality but had apparently little effect on the incidence.

PGI$_2$

PGI$_2$ at the doses tested (25-200μg kg^{-1}) did not seem to have much effect on the STR (Table 3.3) and PTZ (Table 3.4) seizure models, although a dose of 50μg kg^{-1} significantly reduced ($P<0.05$) the incidence of PTZ seizures as well as the mortality. PGI$_2$ (50-200μg kg^{-1}) abolished the mortality following MES and apparently reduced the tonic hindlimb extension.

Table 3,I

Effects of iloprost, PGE, and PGI_2 on strychnine induced seizures in mice

Dose µg kg^{-1} i.p. Iloprost	PGE,	PGI_2	Convulsing animals (%)	Onset of seizures (min) (mean ± sem)	Mortality (%)
-	-	-	56	8,6 ± 0,5	36
63	-	-	0	-	0
32	-	-	0	-	0
16	-	-	20*	14,5 ± 1,5**	10
8	-	-	20*	16,0 ± 3,0**	10
-	200	-	20*	14,0 ± 6,0	20
-	100	-	10*	19,0	0
-	50	-	40	10,3 ± 1,7	25
-	25	-	50	9,8 ± 0,6	25
-	-	200	40	10,3 ± 1,1	30
-	-	100	40	8,0 ± 0,4	30
-	-	50	70	8,9 ± 0,6	50
-	-	25	60	8,3 ± 0,5	40

All the mice were injected with strychnine (1,25 mg kg^{-1} s.c.), *, ** are significantly different from control group at $P < 0,05$ Chi-squared test with Yates correction for continuity, and $P < 0,05$ Student's t-test, respectively. Number of animals in each group 10, except in controls 81.

Table 3,2

Effects of iloprost, PGE_1 and PGI_2 on pentylenetetrazol-induced seizures in mice

Dose µg kg^{-1} i.p. Iloprost	PGE_1	PGI_2	Convulsing animals (%)	Onset of seizures (min) (mean ± sem)	Mortality (%)
-	-	-	76	19,9 ± 1,5	74
63	-	-	20*	29,0 ± 6,0**	10
32	-	-	30*	23,3 ± 0,9	30
16	-	-	30*	25,0 ± 5,2	30
8	-	-	40*	15,5 ± 2,6	40
-	200,0	-	60	28,8 ± 3,8**	40
-	100,0	-	40*	25,3 ± 2,1	40
-	50,0	-	50	29,2 ± 1,8**	40
-	25,0	-	60	20,2 ± 3,0	60
-	-	200,0	70	20,7 ± 3,9	70
-	-	100,0	70	24,3 ± 2,6	70
-	-	50,0	40*	20,8 ± 4,9	40
-	-	25,0	80	18,6 ± 3,0	80

All the mice were injected with pentylenetetrazol (108,0 mg kg^{-1} s.c.), *, ** are significantly different from control group at $P < 0,05$ Chi-squared test with Yates correction for continuity, and $P < 0,05$ Student's t-test, respectively, Number of animals in each group 10, except in controls 50,

Table 3.3

Effects of iloprost, PGE_1 and PGI_2 on maximal electroshock induced seizures in mice

Dose µg kg^{-1} i.p. Iloprost	PGE_1	PGI_2	Convulsing animals (%)	Mortality (%)
-	-	-	55	13
63	-	-	30	0
32	-	-	20*	0
16	-	-	40	10
8	-	-	50	20
-	200,0	-	60	0
-	100,0	-	50	10
-	50,0	-	30	20
-	25,0	-	30	20
-	-	200,0	40	0
-	-	100,0	40	0
-	-	50,0	30	0
-	-	25,0	60	10

All the mice were subjected to maximal electroshock (194 V 50 pps, 200 mS), * is significantly different from control group at $P < 0,05$ Chi-squared test with Yates correction for continuity, Number of animals in each group 10, except in controls 77

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3.4 *A controlled study of the effects of dietary supplementation of mackerel oil on seizure susceptibility in mice*

Enrichment of the diet of the mice with mackerel oil significantly increased their mortality and apparently increased the seizure incidence following STR and PTZ (Table 3.4). The mean weight gain of both control and test groups were similar throughout the feeding period (Table 3.5).

Table 3.4

Comparative susceptibility of control and test mice to strychnine (STR) and pentylenetetrazole (PTZ) seizures after the 10 days feeding on mackerel oil (EPA).

Feed composition	Dose (mg/kg) STR	PTZ	Convulsing Animals (%)	Onset of tonic seiz. (min; mean± S.E.M.	Percentage mortality
Normal feed (N)	1,25	-	60	9,72±0,42	50,00
N + 10% EPA	1,25	-	80	8,92±0,34	80,00*
N + 20% EPA	1,25	-	80	9,12±0,37	80,00*
N	-	135	50	18,80±2,30	50,00
N + 10% EPA	-	135	48	19,08±2,22	48,00
N + 20% EPA	-	135	56	18,78±1,23	56,00

* Significantly different from control at $P < 0,025$ Chi-squared test with Yates correction for continuity.

Table 3.5

Relative weight gain of the control and test mice during the 10-day feeding on mackerel oil (EPA).

Feed composition	Weight Gain (g; mean ± sem) Day 1	Day 5	Day 7	Day 10
Normal feed (N)	0,00	0,70±0,07	1,08±0,12	2,00±0,11
N + 10% EPA	0,00	0,85±0,05	0,98±0,10	1,70±0,12
N + 20% EPA	0,00	0,83±0,09	1,15±0,17	2,00±0,13

3.5 *Cyclic AMP levels in the mouse brain tissue during pentylenetetrazole activity and in the presence of PGE, and iloprost*

Pentylenetetrazole induced an apparent increase in cyclic AMP in the mouse brain 15 min post-drug administration. PGE_1 (200 µg/kg) potentiated the action of pentylenetetrazole on cyclic AMP levels significantly ($P<0.05$) when compared to the control group. The increases in cyclic AMP induced by low dose of PGE_1 (50 µg/kg) and iloprost (50 µg/kg) were however not statistically significant (Table 3.6).

Table 3,6

Cyclic AMP levels in the mouse brain tissue during pentylenetetrazole activity and in the presence of PGE, and iloprost,

Batch No,	Drugs and doses (mg/kg)				Cyclic AMP levels (pmol/mg protein; mean ± S,E,M)
	Iloprost	PGE,	*Saline*	PTZ	
1	-	-	0,2ml	-	2,48 ± 0,28
2	-	-	-	100	2,83 ± 0,33
3	-	0,05	-	100	3,94 ± 0,45*
4	-	0,10	-	100	3,76 ± 0,88
5	0,05	-	-	100	3,03 ± 0,65

n = 5

* = significantly different from control group at P < 0,05 Student's t-test

3.6 *Effects of PGE, and oxytocin on the isolated rat uterus*

(i) Qualitative difference between the contractile effects of PGE, and oxytocin on the isolated rat uterus.

PGE, ($1.4x10^{-6}M$) and oxytocin ($5x10^{-7}M$) induced rapid contractions of the isolated rat uterus with two peak contractions and an intermediate partial relaxation. The partial relaxation observed was more marked for PGE, than for oxytocin (Fig. 5a & b). Absolute velocity for contraction and relaxation was greater for oxytocin and the contraction relaxation cycle was longer when compared to PGE, (Table 3.6)

(ii) Influence of piroxicam and IBMX on the contractile actions of PGE, on the isolated rat uterus

PGE, stimulated the isolated rat uterus to *contract in a concentration dependent manner* (Fig. 6a). PGE, induced uterine contractions in which two peaks appeared within one minute contact time

with the drug.

Low concentrations of piroxicam (1×10^{-7} to 1×10^{-5}M) did not have much effect on the PGE_1-induced contractile responses. At higher concentrations, piroxicam (1×10^{-4} to 1×10^{-3}M) progressively diminished the PGE_1-induced responses. The 2-peak response induced by PGE_1 was reduced to only 1-peak (Fig. 6b). The contraction/relaxation velocity ratio was markedly reduced by piroxicam (Table 3.7).

IBMX also reduced the PGE_1-induced contraction of the isolated rat uterus (Fig. 6c). In addition, IBMX attenuated the contraction velocity and increased the relaxation velocity of the uterine smooth muscle following PGE_1 stimulation (Table 3.7)

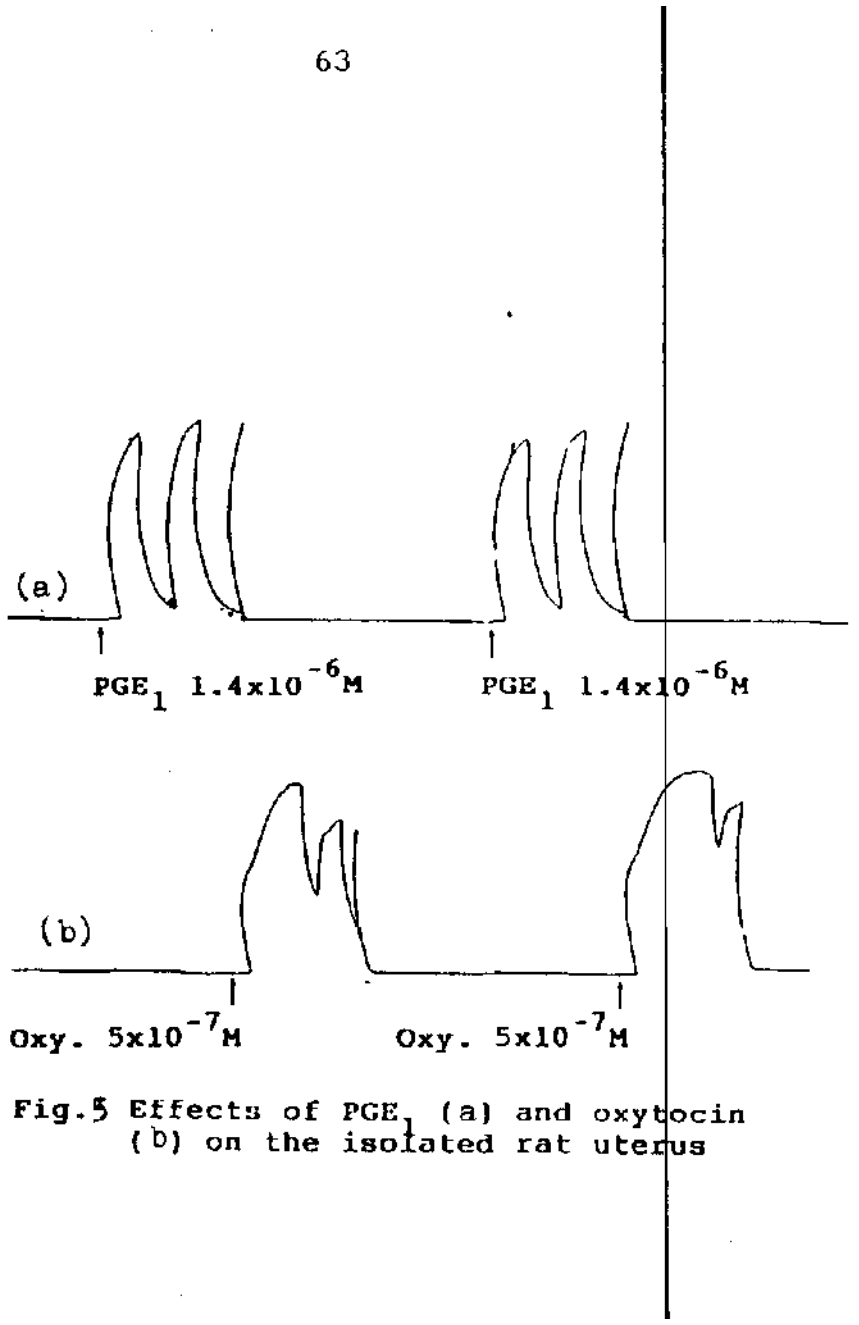


Fig.5 Effects of PGE_1 (a) and oxytocin (b) on the isolated rat uterus

Table 3.7

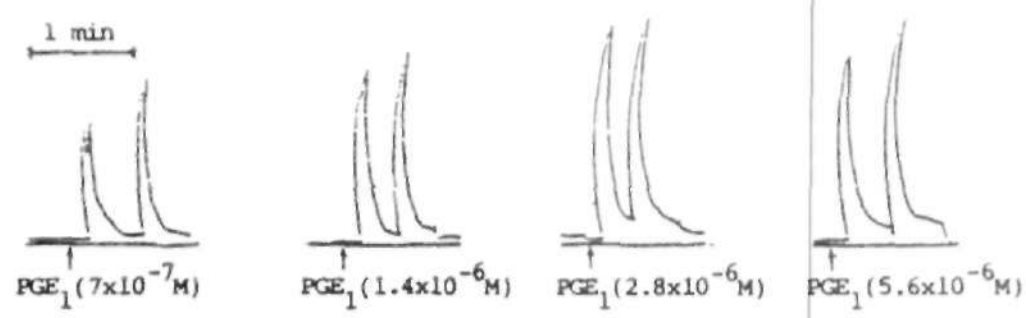
Contractile parameters for PGE, and oxytocin.

Drug (mol/l)	Ampl. of contraction (mV)		Abs. vel. contr. / Abs. vel. relax.
	1	2	(Mean ± S.E.M.)
Spont. contr.	1299	-	0.62±0.04
PGE_1 (1.4×10^{-6}M)	1888	1954	0.94±0.18
Oxy. (5×10^{-7}M)	2422	1877	1.27±0.10

n = 3

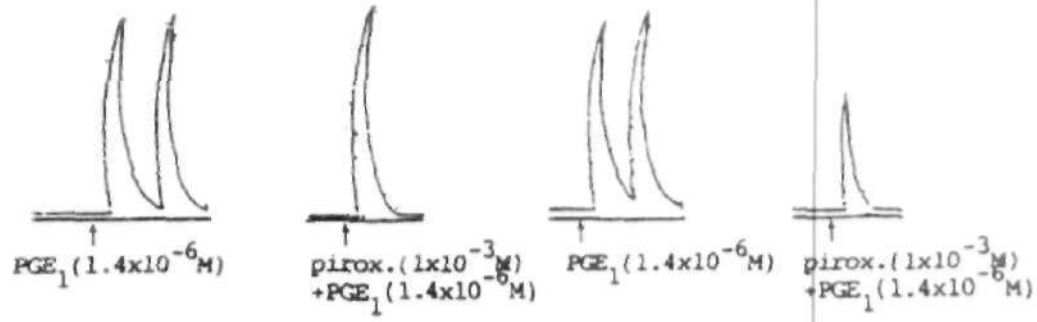
Fig. 6
Effects of piroxicam and IBMX on the contractile action of PGE_1 on the isolated rat uterus.

(a)



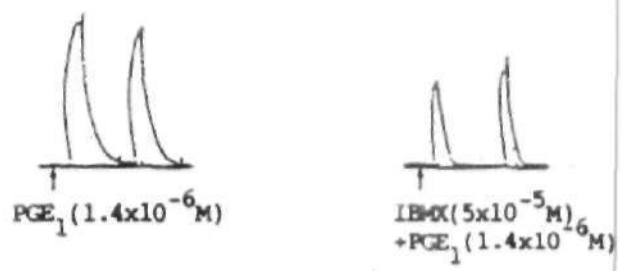
Effect of PGE_1 on the isolated rat uterus.

(b)



Effect of piroxicam (pirox) on the contractile action of PGE_1 on the isolated rat uterus.

(c)



Effect of isobutylmethylxanthine (IBMX) on the contractile action of PGE_1 on the isolated rat uterus.

Table 3,8 Influence of piroxicam (Pirox) and isobutylmethylxanthine (IBMX) on the contractile responses of the isolated rat uterus to PGE,

Drug (M)	Amplitude of Contr,(mV) 1	2	Max, contr,vel Max, relax,vel, (mV/sec) 1	2	Contr, vel,/ relax vel, 1	2
PGE,	2935	3112	920;321	888;381	2,9	2,3
Pirox, (10^{-4}) + PGE1	3090	3220	929;481	838;562	1,9	1,5
PGE,	2844	3040	935;366	969;494	2,6	2,0
Pirox, ($2x10^{-4}$) + PGE,	3058	2844	907;433	977;496	2,1	2,0
PGE,	2352	2496	609;245	628;393	2,5	1,6
Pirox, ($5x10^{-4}$) + PGE,	2622	-	534;264	-	2,0	-
PGE,	2272	2484	563;197	638;380	2,9	1,7
Pirox, ($1x10^{-3}$) + PGE,	1503	-	278;349	-	0,8	-
PGE,	1035	955	193;187	212;156	1,0	1,4
IBMX ($1x10^{-6}$) + PGE,	982	952	182;213	196;151	0,9	1,0
PGE,	1278	1193	284;191	277;148	1,3	1,9
IBMX ($1x10^{-5}$)	1064	1034	207;282	240;258	0,7	1,1
PGE,	1527	1409	316;194	305;190	1,6	1,6
IBMX ($2x10^{-5}$) + PGE,	1155	1240	215;393	306;358	0,6	0,9
PGE,	1527	1409	326;194	305;190	1,6	1,6
IBMX ($5x10^{-5}$) + PGE,	926	1208	137;314	265;460	0,4	0,6

* Abbreviations; Contr, = contraction; Relax,= relaxation; Max, = maximum; Vel, = Velocity; mV = millivolt; sec, = second; M =Molar,
Concentration of PGE, used in all the tests was $1,4x10^{-6}$M, 1 and 2 represent values for 1st and 2nd peaks, respectively, *Means (n = 3) are given,

3.7 *Effects of PGE, on isolated rat uterus in the presence of verapamil*

PGE, produced dose-dependent responses in normal De Jalon, high potassium De Jalon, and high calcium De Jalon solutions. The normal responses of the isolated rat uterus to PGE, were increased in the presence of high calcium concentration whereas in the high potassium De Jalon, the responses were decreased (Fig. 7a).

PGE, produced dose-dependent responses in normal De Jalon solution which were blocked by high verapamil concentration ($1x10^{-5}M$). On the other hand low concentration ($2x10^{-7}M$) of verapamil failed to inhibit these responses (Fig. 7b)

High verapamil concentration ($1x10^{-5}M$) blocked the responses to PGE, in the presence of high calcium and low concentration of verapamil ($2x10^{-7}M$) reduced the responses to PGE, in high calcium De Jalon solution (Fig. 7c).

The responses of the isolated rat uterus to PGE, in high potassium De Jalon solution were further reduced by both high ($1x10^{-5}M$) and low ($2x10^{-7}M$) concentrations of verapamil (Fig. 7d)

Fig. 7a
Effect of PGE_1 on the isolated rat uterus suspended in normal De Jalon solution (○—○), De Jalon with double calcium (4.87 mM Ca ;●--●) and potassium depolarised De Jalon solution (100 mM K ;■--■)

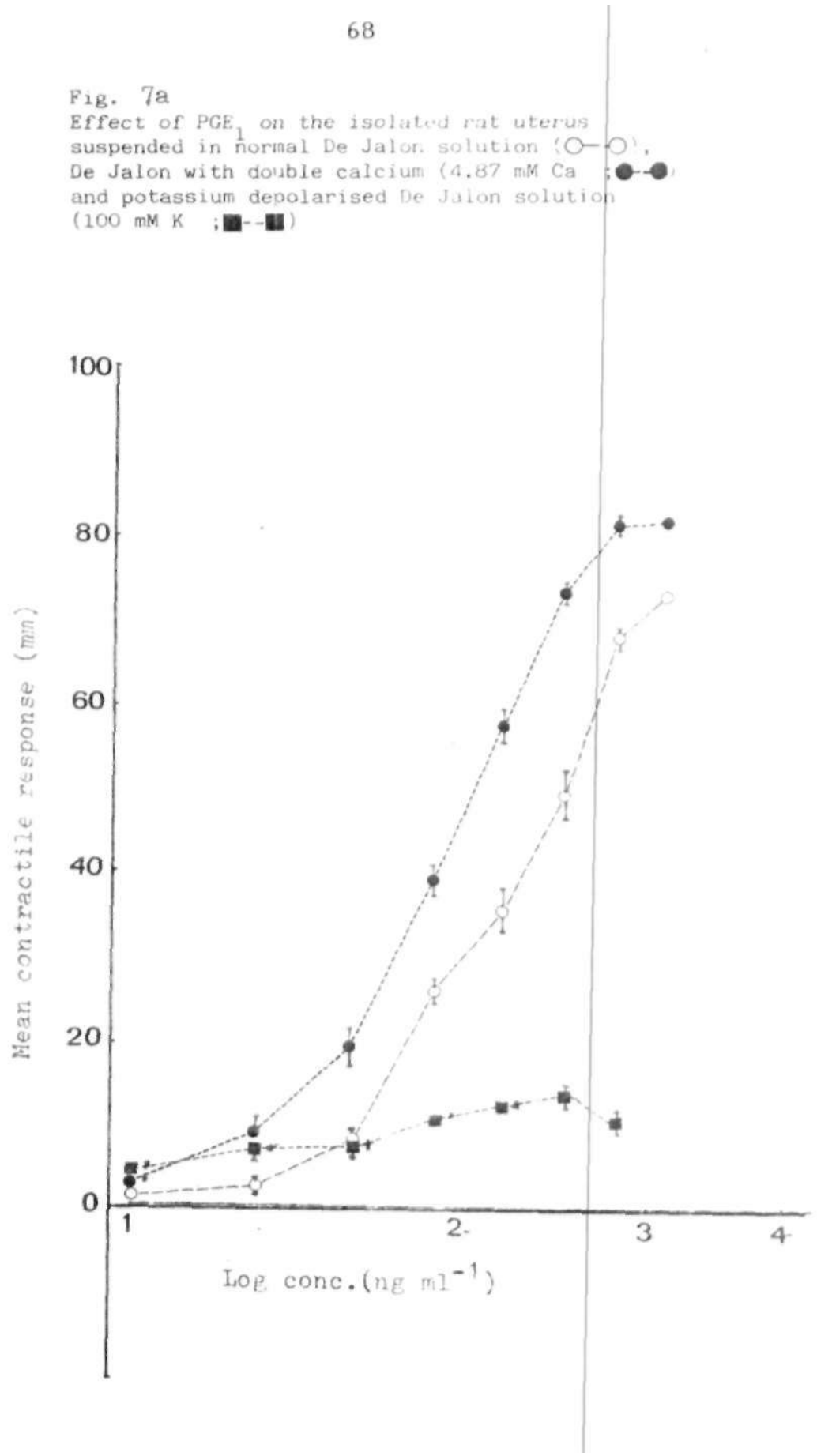


Fig. 7b
Effect of PGE_1 on the isolated rat uterus suspended in normal De Jalon solution (○–○), and in the presence verapamil, low (2×10^{-7} M; ◇–◇) and high (1×10^{-5} M ;◆–◆) concentrations.

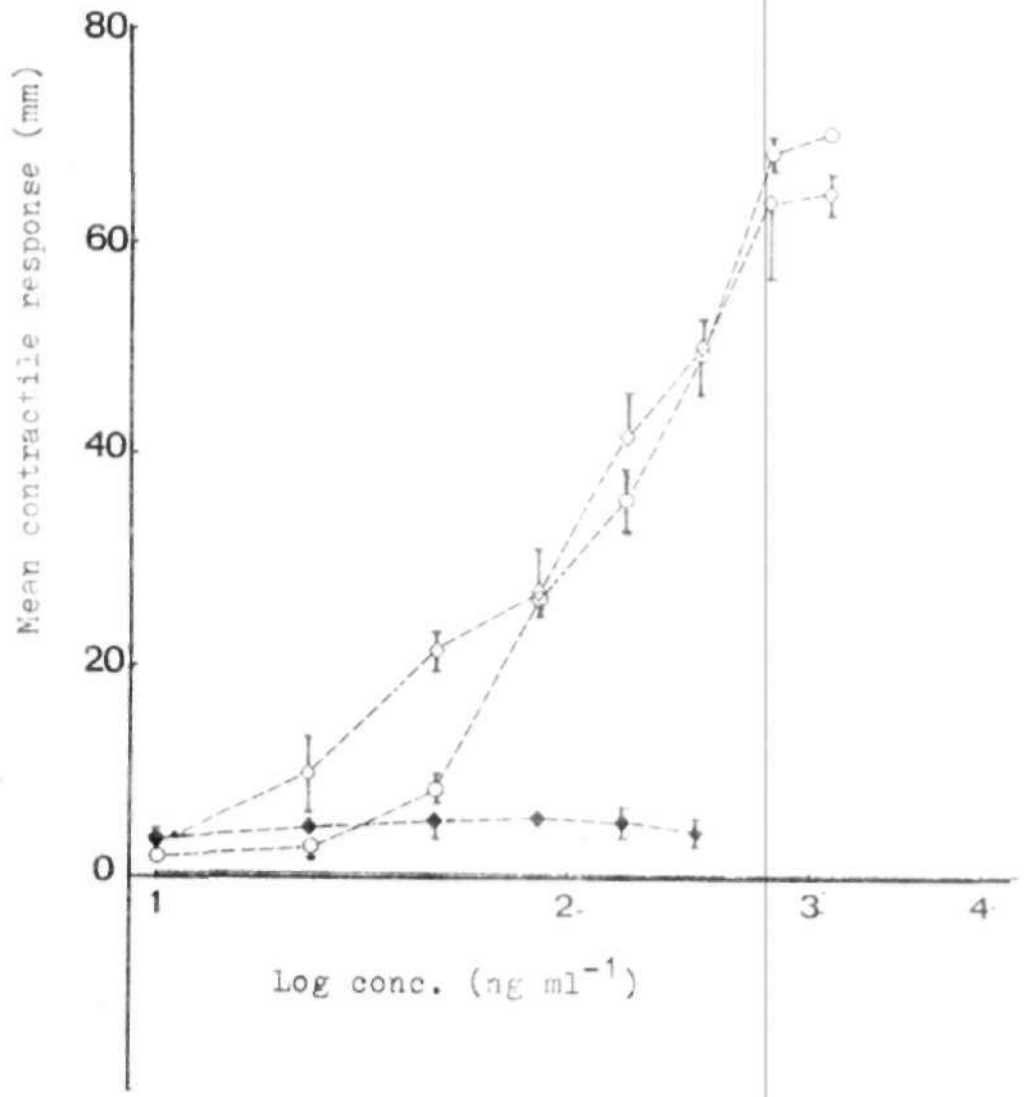


Fig. 7c
Effect of PGE_1 on the isolated rat uterus suspended in De Jalon with double calcium concentration (4.87 mM) and in the presence of verapamil, low (2×10^{-7} M;◇-◇ and high (1×10^{-5} M;◆-◆ concentrations.

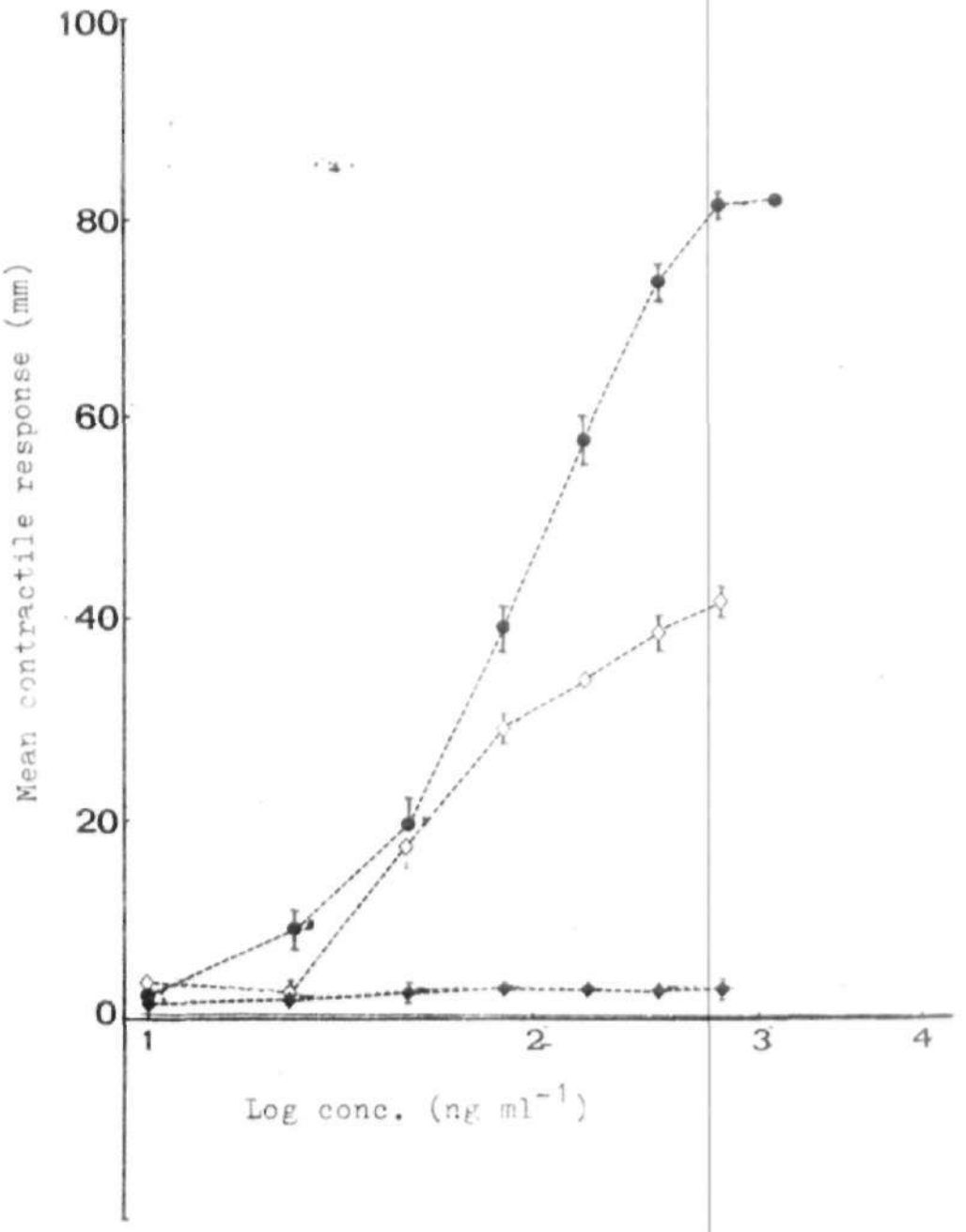
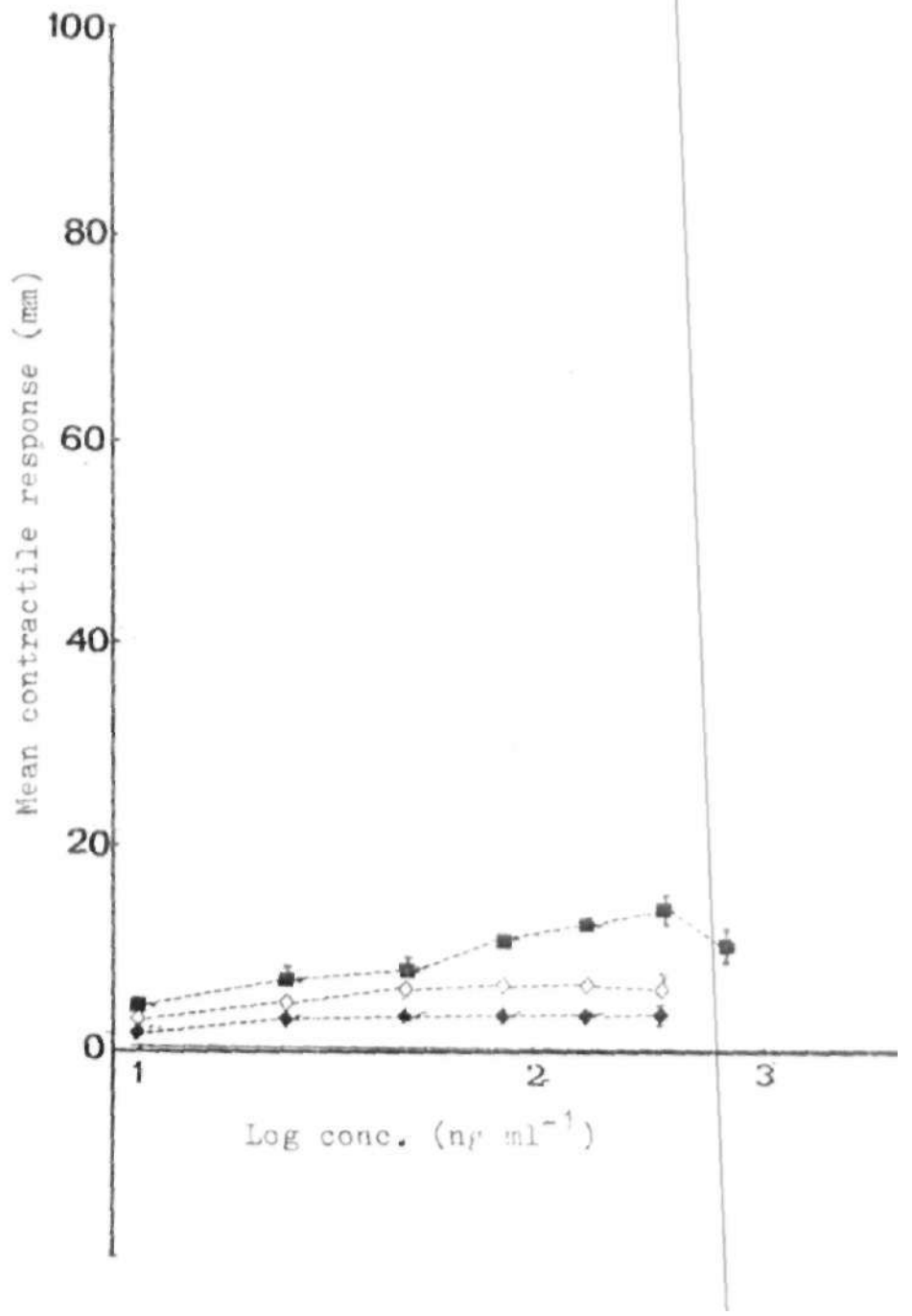


Fig. 7d
Effect of PGE_1 on the isolated rat uterus suspended in potassium depolarised De Jalon solution (100 mM K ;■-■) and in the presence of verapamil, low (2×10^{-7} M;◇-◇) and high (1×10^{-5} M ;◆-◆) concentrations.



3.8 *Cyclic AMP assay from the uterine smooth muscle*

PGE, significantly increased (P<0.001) cAMP levels in the isolated rat uterus (Table 3.8). The peak effect was registered at 20 seconds post drug administration. There was also an immediate rise in the cyclic AMP level at 5 seconds which reduced sharply at 10 seconds before the sharp peak at 20 seconds. Cyclic AMP level gradually reduced to the control level (c) within 1 minute (Fig 8).

Table 3.9

PGE,-induced changes in cyclic AMP content of the rat uterine tissue with time.

Batch No.	Freeze clamping time (sec)	cAMP concentration pmol/mg protein (mean ± SEM)
1	0.0	16.18 ± 1.2
2	5.0	26.92 ± 7.4
3	10.0	16.16 ± 2.2
4	20.0	37.34 ± 4.2*
5	30.0	28.92 ± 4.9*
6	40.0	18.70 ± 3.4
7	50.0	13.84 ± 2.3
8	60.0	15.33 ± 1.8

n = 5

* = are significantly different from control at $P < 0.001$

Student's t-test

CHAPTER FOUR

4 DISCUSSION

4.1 **Prostaglandins and the CNS**

Catalepsy is a depressed state, characterised by decreased DA in the brain of animals, and the EEG is a useful tool in monitoring the electrical activity of the brain. In this study, iloprost, a new structurally modified chemically stable carbacycline analogue of PGI_2, synchronised the EEG (Fig. 3a & b) and induced profound catalepsy in the rats (Fig. 4). PGE_1 also induced catalepsy in the rats. The cataleptic effects of iloprost and PGE_1 were completely abolished by amphetamine. These results are consistent with those of Horton (1964) who was the first to produce evidence for the depressant action of PGs on the CNS. Prostaglandins have been reported to produce stupor in cats, sedation in young chicks and mice. (Holmes and Horton, 1968). Brus *et al.* (1983) have also observed that PGs can potentiate or reduce neuroleptic-induced catalepsy and antagonise mouse climbing behaviour (Brus *et al.* 1982). These effects are probably produced

through DA receptor blockade. In this experiment, pimozide, a specific DA receptor antagonist (Anden *et al.*, 1970) also induced catalepsy. Both PG and pimozide-induced catalepsies were antagonised by amphetamine, providing further evidence that the depressant effects of both iloprost and PGE, might be produced via the DA system. Thus DA receptor blockade may underlie the behavioural and EEG effects of PGs. However, Costall and his group (1985) have proposed that the abilities of PGs to inhibit dyskinesias differentially may be due to an action at a site beyond the DA receptors. In addition, Czlonkowski and Herman (1980), using receptor labelling assays, found that PGE_1, E_2, D_2 and $F_{2\alpha}$ could not displace ^{3}H-spiperone from striatal DA receptors *in vitro*. These results indicate that PG actions may not necessarily be mediated through a direct action on DA receptors, but such actions might be remotely produced at a site that may be in close functional association with the DA receptors.

The effects of iloprost, PGE_1 and PGI_2 on epileptic seizure mechanisms were also studied and the results show that these drugs possess anticonvulsant activities against both chemically and electrically - induced seizures in mice (Table

3.1, 3.2 & 3.3). These results are similar to those of other workers (Duru and Turker, 1969; Gilmore and Shaik, 1972; Rosenkranz and Killam, 1978) who have consistently reported that PGE_1 and PGE_2 given prior to a variety of convulsive agents, reduce the severity of seizures, prolong their onset and reduce the mortality. In this experiment iloprost and PGE_1 significantly reduced the incidence of seizures ($P<0.05$) induced by STR and PTZ, prolonged the onset and reduced the mortality. Similarly, iloprost profoundly ($P<0.05$) reduced MES-induced seizures and decreased the mortality. Iloprost, PGE_1 and PGI_2 also antagonised the mortality following MES seizures. The effects observed in this experiment are probably of central origin as they occurred at doses which elicit behavioural responses.

There is a good correlation between the ability of a drug to inhibit MES in rodents and its effectiveness in seizures in humans (Browning and Nelson, 1985). Drugs which abolish tonic hind-limb extension in MES seizures may be effective against generalised tonic-clonic epilepsy, while those that elevate PTZ seizure threshold typically suppress absence seizures. Strychnine was employed in this study to further explore the possible

mechanism of PG actions. Epilepsies are a group of diverse central disorders with a common symptom of seizures, which tend to be recurrent. The cellular hallmark of the abnormal discharges during epilepsy is the occurrence of prolonged neuronal depolarisation (MacVicar and Dudek 1981; Pumain 1981). Both synaptic and nonsynaptic membrane currents seem to be involved (Johnston and Brown 1981; Gutnick *et al.*, 1982; Gamaniel *et al.*, 1988)

The main difficulty in interpreting the results of this experiment is that the different models of epilepsies employed have other, perhaps more specific sites of action on a variety of membrane, transmitter or enzyme systems. For example, PTZ has been suggested to cause decrease in neuronal recovery time (Esplin and Zablocka-Esplin, 1969), so that a single stimulus may lead to repetitive discharges. PTZ selectively antagonises γ-aminobutyric acid (GABA)-mediated post-synaptic inhibition in cultured mouse spinal cord neurons (MacDonald and Baker, 1977) and attenuates cholinergic inhibitory post-synaptic potentials without marked effects on excitatory post-synaptic potentials in the same neurons. PTZ may also have direct effects on membrane properties leading to negative resistance in the neurons, increasing

potassium permeability and neuronal firing. Furthermore, PTZ seizures has been found to increase cyclic AMP and cyclic GMP levels in mouse brain which correlates well with the convulsive activity (Ferrendelli and Kinscherf, 1977; Ferrendelli, 1983). The anticonvulsant actions of iloprost and PGE, against PTZ may therefore be related to a direct neuronal mechanism leading to prolongation of the neuronal recovery time. This speculation is supported by earlier observations on the actions of PGE, on spinal reflexes (Khairallah and Wilcoxon, 1967) and neuromuscular junction (Duda *et al.* 1968). PTZ convulsive threshold correlates well with increased GABA levels in brain and in synaptosomes. Experimental and clinical data suggest that central GABAergic system is involved in the pathogenesis of epilepsy and may play a role in the antiepileptic mechanism (Loscher 1981). It is therefore possible that GABA might have contributed to the observed effects of iloprost and PGE, against PTZ. Indeed, evidence presented by Rosenkranz and Killam (1979) also indicates that the anticonvulsant action of the E series PGs may be coupled with GABAergic mechanism. Moreover, PGE_2 which is also believed to act via a similar mechanism to PGE, in the CNS has been found to increase brain GABA levels by 30

- 50% (Rosenkranz *et al.* 1980). However, GABA is present in many cells including glial cells, and in neurons which do not use this amino acid as a neurotransmitter (Balazs *et al.*, 1970). Thus, the cellular compartmentation of GABA makes it difficult to interprete the correlation between overall elevation in the brain and neuropharmacological effects such as anticonvulsant actions. Cyclic nucleotides have also been considered as part of the molecular mechanisms in the pathogenesis of epilepsy, and because they can alter the synaptic membrane properties involved in ion fluxes, changes in their metabolism have been thought to be relevant in neurological diseases (Cramer *et al.*, 1979; Palmer *et al.*, 1979). Cyclic AMP may act at the membrane to alter ion permeability as well as having other actions. Increased cyclic AMP levels in the brain may have an antiepileptic effect and perhaps have some role in the mechanisms inhibiting seizure spread and/or duration of seizures. Most drugs which inhibit the PTZ-induced increase in cyclic AMP facilitate seizure activity, while those that do not depress cyclic AMP accumulation have little effect or decrease seizure activity (Ferrendelli, 1983). It is significant in this study that pentylenetetrazole

caused an increase in yclic AMP levels in the mouse brain when compared to controls. PGE_1 and Iloprost potentiated the effects of pentylenetetrazole on mouse brain cyclic AMP levels. In the other study cited above (Table 3.2) PGE_1 and Iloprost reduced the incidence of pentylenetetrazole seizures in mice and delayed the onset at the same doses that potentiated pentylenetetrazole induced increase in cyclic AMP levels. It is possible therefore that these prostaglandins reduced the severity of pentylenetetrazole-induced seizures in the mice via the elevation of brain cyclic AMP levels thus leading to the attenuation of seizures. These results support those of Ferrendelli (1983) who also proposed that increased cyclic AMP levels in the brain may have anticonvulsant effects and perhaps have some role in the mechanisms of seizure spread and/or duration.

Iloprost is a stable analogue of PGI_2 which has been synthesised recently and has similar actions on different biological preparations (Schror *et al.*, 1981a & b; Ercan *et al.*, 1983). It is generally accepted that iloprost, PGE_1 and PGI_2 activate adenyl cyclase with the consequent elevation of intracellular cyclic AMP. This

mechanism is also apparent in their effectiveness against platelet aggregation (Schillinger and Losert, 1980). PGE_1 and E_2 (1mg kg^{-1} i.v.) have been shown to lead to 60-70% increase in the cyclic AMP content of the rat brain (Stamm and Wellmann, 1973). PG-sensitive accumulation of cyclic AMP has also been demonstrated in tissue slices from most areas of the brain (Dismukes and Daly, 1975).

STR increases the excitability of the spinal cord by blocking the supraspinal inhibition selectively through glycine. The selectivity between STR and glycine is high, and STR blocks selectively glycine neurons without decreasing the effects of GABA (Johnson *et al.*, 1970). The nearly complete abolition of STR-induced seizures by PGs in this study agrees with earlier results (Duru and Turker, 1969) and threby suggesting that the anticonvulsant action of PGs may be attributable to glycine receptor activity. The relatively high potency of iloprost may be explained by its high chemical stability. However, the activity of the PGs against other models of laboratory seizures does suggest that similar molecular or cellular events might underlie the observed effects.

Iloprost and PGE_1 were more potent in blocking threshold seizures induced by PTZ and STR than in preventing MES seizures. Thus their anticonvulsant activities may be more related to the threshold mechanism than the spread of seizures. However, these PGs (iloprost at high doses and PGE_1 at low doses) also seem to reduce MES seizure incidence and mortality. At high doses, iloprost, PGE_1 and PGI_2 antagonised the mortality following MES and in this respect, PGI_2 was very effective. PGI_2 is unstable and is rapidly hydrolysed to biologically inactive 6-keto$PGF_{1\alpha}$. Another PG, 6-ketoPGE_1, has attracted recent attention because of its formation *in vitro* from PGI_2 and $PGF_{1\alpha}$ in human platelets (Hoult *et al.*, 1981). 6-ketoPGE_1 shares many of the biological activities of PGI_2 and is stable under physiological conditions. In many tests, it has also been found to be at least as potent as PGI_2 (Berry *et al.*, 1984). Therefore, 6-ketoPGE_1 is a metabolite which might have contributed to the observed effects of PGI_2. These findings are similar to those reported by Rosenkranz and Killam (1978) who also found that PGE_1 and PGI_2 inhibited tonic convulsions induced by MES. These workers showed that PGI_2 in higher doses (250-2000$\mu g\ kg^{-1}$) than those used in this experiment failed to block the incidence of tonic

hindlimb extension but decreased their duration. PGE_1 failed to inhibit the incidence of tonic hindlimb extension induced by MES in the present study. Although the mortality was apparently reduced by PGE_1 at high doses, low doses reduced the incidence but increased the mortality. This phenomenon might be attributable to the effects of PGE_1 or its metabolites on other organ systems of the body. Earlier, Poddubiuk (1976) had also observed that PGE_1 did not have much effect on the incidence of seizures following MES but shortened the duration of tonic hindlimb extension. The results differ from those of Holmes and Horton (1968) who have reported that 1000$\mu g\ kg^{-1}$) of PGE_1 decreased MES-induced tonic convulsion in mice by 50%. The disparities in different studies can be explained by the various doses and other experimental conditions used.

It is becoming clear that nonsynaptic mechanisms may play a major role in seizures, and comparison of various experimental epilepsies reveals a homogenous sign of epileptiform activity at the cellular level (Heineman and Haman, 1986). Generally, there is a lack of clinical correlates to the cyclic nucleotide fluctuations observed under experimental situations using cellular and

animal models. Nevertheless, there appears to be a negative correlation between cyclic AMP accumulation in the brain and seizure activity with cyclic AMP usually having an inhibitory effect on central neuronal systems.

The effects of endogenous synthesis and release of PGs and their precursors must be considered when studying their exogenous actions. The type of essential fatty acid in the diet strongly influences the fatty acid composition of tissue and serum lipids (Bragdon and Karmen, 1961; Fischer and Weber, 1984). Changes in the fatty acid composition of the cell membranes may also be associated with changes in fluidity, carrier-mediated transport and the activity of certain membrane-bound enzymes (Spector and Yorek, 1985). For example, the activity of adenylate cyclase enzyme can be affected by changes in the phospholipid fatty acyl chains and polar head groups and also by changes in the cholesterol content of the plasma membrane. Furthermore, dietary cod liver oil supplementation with a consequent increase in n-3 fatty acids in the membrane phospholipids of rat atria has been found to be associated with a decrease in the formation of cyclic AMP both in the basal state and in

response to NA stimulation and these biochemical changes are accompanied by marked changes in physiological performance (Laustiola *et al.*, 1986).

In this experiment, dietary mackerel oil supplementation apparently increased the seizure incidence following STR and PTZ and increased the mortality in the mice significantly ($P<0.05$). Many studies have documented that the dietary marine lipids alter the production of eicosanoids in human and experimental animals. Incorporation of large quantities of n-3 polyunsaturated fatty acids (abundant in marine lipids) into tissues of humans and experimental animals may lead to synthesis of eicosanoids derived from n-3 fatty acids, primarily eicosapentanoic acid (EPA), and may suppress the production of eicosanoids derived from AA (Robinson, 1985). The observed effects might have therefore resulted from enrichment of the cell membrane with EPA, with the consequent increase in the production of PGs of the 3 series in the mice. It is also possible that changes in the membrane fluidity, carrier mediated transport or the activity of some membrane-bound enzymes for example, adenylate cyclase, might also underlie the effect of mackerel oil supplementation in the

mice. Whether this finding that mackerel oil supplementation augments seizures also applies to human subjects is uncertain, but mackerel oil supplementation has been found to affect some physiological functions in man, leading to decrease in blood pressure, and response of blood pressure to NA (Laustiola *et al.*, 1986).

Calcium dependent mechanisms also appear to play a major role in generating seizure responses. It has been shown that in *in vitro* hippocampal slices, *decreasing the extracellular* Ca^{++} *concentration* can induce synchronised neuronal activity that can spread by nonsynaptic mechanisms (Jefferys and Hass, 1982; Konnerth *et al.*, 1984; Taylor and Dudek, 1984). Cyclic AMP is thought to mediate the positive inotropic effect of catecholamines on the myocardium (Drummond and Severson, 1979) probably by producing an increase in the average number of functional Ca^{++}channels per cell leading to an increased slow-inward-current of Ca^{++} (Bean *et al.*, 1984). Cyclic AMP also enhances relaxation of the heart muscle by increasing Ca^{++} uptake into the sarcoplasmic reticulum (Katz, 1979). Cyclic AMP and its close association with membrane ion transfer, especailly with Ca^{++}, may therefore subserve in the seizure phenomenon and possibly

forms the basis of the anticonvulsant actions of PGs. Indeed verapamil, nifedipine and other Ca^{++} antagonist also have the property to suppress seizure generation (Louvel *et al.*, 1986; Speckmann *et al.*, 1986)), by moderating Ca^{++} entry into the CNS for the classical Ca^{++} currents as well as having other actions.

4.2 Prostaglandins and the uterine smooth muscle

Cyclic nucleotides are thought to act as second messengers to PGs in many cell types, and it is possible that most of PG actions on biological systems may be shown to be mediated through changing levels of cyclic nucleotides (Samuelsson *et al.*, 1975; Uotila and Vapaatola, 1984). Biological membranes and their interaction with Ca^{++} may also be targets for PGs. For example, modification of membrane permeability to Ca^{++} may account for the effect of PGs on platelet aggregation and contractility of cardiac and smooth muscles. PGE_1 is a potent compound in stimulating the uterus to contract. In this study, PGE_1 induced a dose dependent contraction of the isolated rat uterus in which two peak contractions appeared within one minute contact time (Fig. 6a). A comparison of this mode of contraction to that of oxytocin revealed that, whereas both drugs induced rapid contractions with two peaks and an intermediate partial relaxation, the partial relaxation phase was more pronounced for PGE_1 than oxytocin (Fig. 5a & b). Furthermore, PGE_1 was found to cause a time dependent increase in cyclic AMP levels in the isolated rat uterus

significantly (Table 3.8). PGE_1 has also been shown to increase cyclic AMP levels in the rat heart and to activate phosphorylase (Malik and McGiff, 1976; Metsa-Ketela, 1981). On the other hand, it has been observed that oxytocin may increase cyclic GMP levels in the rat uterine smooth muscle with no effect on cyclic AMP level (Goldberg *et al.*, 1973; Kuehl *et al.*, 1973). Cyclic AMP may therefore contribute to the apparent difference between the contractile response of the isolated rat uterus to oxytocin and PGE_1 by enhancing the partial relaxation in response to the latter. In addition, the absolute contraction/relaxation velocity for oxytocin-induced response was much higher than that of PGE_1 indicating a larger relaxation component for PGE_1 compared to that of oxytocin (Table 3.6), thus supporting the speculation that cyclic AMP might contribute to the relaxation mechanism. It has been suggested by Metsa-Ketela (1981) that at least in the cardiac muscle, cyclic AMP may not mediate the positive inotropic effect of PGE_1 and I_2 but may rather be responsible for increased relaxation, which might prevent the full development of tension. However, there is no direct evidence from this study to permit speculation on the exact role of cyclic AMP in the

response elicited by PGE_1, since the peak contraction and relaxation patterns did not correlate well with the changes in cyclic AMP levels shown within one minute. Further support for the above speculation however comes from the observation that IBMX, a potent phosphodiesterase inhibitor (Daly, 1977) antagonised PGE_1 induced uterine contractions (Fig. 6c) by attenuating the contraction velocity and increasing the relaxation velocity of the uterine smooth muscle following PGE_1 stimulation (Table 3.7). These results are generally consistent with those of Klein and Levey (1971) who have observed that PGE_1 can stimulate the membrane bound adenylate cyclase to produce cyclic AMP from its precursor ATP. In this study, evidence has also been obtained which tend to suggest that piroxicam, a nonsteroidal anti-inflammatory drug (NSAID) might antagonise PGE_1 effects on the isolated rat uterus by enhancing relaxation via the cyclic AMP system. It is believed that inhibition of PG synthesis through the cyclo-oxygenase may account for most of the therapeutic as well as adverse effects of NSAID (Vane, 1971; Ferreira and Vane, 1979).

Ca^{++} channel antagonists are believed to exert their effects primarily at the potential dependent channels where they inhibit Ca^{++} influx (Fleckenstein, 1983). It is significant in this study that low verapamil concentration ($2x10^{-7}M$) did not block the contractile actions of PGE, in normal De Jalon solution but reduced the responses to PGE, in the presence of high Ca^{++} and high K^{+}. High verapamil concentration also blocked responses to PGE, in normal and both high Ca^{++} and K^{+} De Jalon solutions. PGE, produces contractions of the smooth muscles by interacting with specific discrete receptors (Paton and Daniel, 1967). It is apparent from this study therefore that PGE, may not have a major action through potential dependent channels, but may increase Ca^{++} influx via receptor operated channels as well as being able to mobilise intracellular Ca^{++} and thus differing from oxytocin. These results are similar to those of Barnes and Senior (1985) who also found evidence that $PGF_{2\alpha}$ may induce uterine contraction by increasing Ca^{++} influx through the receptor operated channel as well as being able to release intracellular Ca^{++}.

On the isolated rat and guinea-pig uteri, PGE, has been found to have two effects; one is slow direct

contraction which can be modified by increase in the concentrations of Ca^{++}, Mg^{++} and K^{+} ions and a latent response manifested by increased response to other stimulating agents, which cannot be modified by changes in Ca^{++}, Mg^{++} or K^{+} (Hall and Pickles, 1966). It is therefore tempting to suggest from this study, that the direct effect is probably due to the interaction of PGE_1 with its receptors on the cell membrane leading to Ca^{++} influx via the receptor operated channels while the latent effect might result from the activation of adenylate cyclase and the subsequent events due to increased cyclic AMP levels intracellularly. Therefore, although it has been suggested that increased PG production by oxytocin receptor activation may be of major importance in normal labour, (Riemier *et al.*, 1986; Chan, 1980; Roberts *et al.*, 1976), PG production probably only supports the contractile response to oxytocin via the latent cyclic AMP related mechanism. In support of this is the observation that indomethacin (a PG synthesis inhibitor) does not prevent the contractile responses to oxytocin (Dubin *et al.*, 1975) and parturition is difficult to induce premature with exogenous oxytocin but can be more readily induced by PGs.

4.3 CONCLUSIONS

The mechanism by which PGs produce their central actions still remains obscure. Among the problems are the facts that intracerebrally administered PGs may disperse throughout the entire brain, eliciting multiple responses, while systemically administered PGs are known to act on a variety of organs, thus making it difficult to differentiate between their central and peripheral actions. Nevertheless, the results of this study do suggest that the depressant as well as the anticonvulsant activities of iloprost, PGE, and PGI_2 might involve a common basic mechanism possibly involving cyclic AMP and cell membrane interactions. Such mechanism may also be operated through a site remote to, but in close functional link with the DA receptors. Iloprost may be a useful tool in the investigation of this mechanism.

On the isolated rat uterus, there appears to be an obvious qualitative difference between the responses elicited by PGE, and oxytocin. PGE, may not have a major action through the potential dependent Ca^{++} channels, but may act via receptor operated channels as well as being able to

mobilise intracellular Ca^{++}, unlike oxytocin. PGE_1 also produced a significant ($P<0.001$) time related increase in cyclic AMP levels in the isolated rat uterus which seems to have contributed to the apparent difference in the uterine responses to PGE_1 and oxytocin in this experiment, by enhancing relaxation. Piroxicam, a nonsteroidal antiinflammatory drug, may antagonise PGE_1 effects on the isolated rat uterus by enhancing relaxation through the cyclic AMP system, probably by inhibiting phosphodiesterase mediated breakdown of cyclic AMP.

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