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PERIPHERAL ANALGESIA: MECHANISM OF THE ANALGESIC ACTION OF ASPIRIN LIKE DRUGS AND OPIATE-ANTAGONISTS

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1 Prostaglandins released by tissue injury sensitize nociceptors and produce hyperalgesia.

2 Aspirin-like drugs inhibit prostaglandins I_2 and E_2, synthesis, which explains their anti-algic effect.

3 The anti-algic effect of aspirin-like drugs in carrageenin-induced rat paw inflammation may involve a central component.

4 Prostaglandin E_2-induced hyperalgesia, once established, is not relieved by systemically administered drugs.

5 Prostaglandin-induced hyperalgesia is possibly a cyclic adenosine, 3′,5′-monophosphate C^{2+} dependent process.

6 Morphine, enkephalins, opiate antagonists and cyclic guanosine 3′,5′-monophosphate have a peripheral analgesic effect in the prostaglandin hyperalgesia test.

7 Morphine may produce peripheral analgesia by inhibiting adenylatecyclase activity at the nociceptors.

Introduction

HYPERALGESIA, the sensitization of nociceptors to mechanical or chemical stimulation, is a basic event in inflammatory pain (Keele & Armstrong, 1964; Dalessio, 1972).

The somatosensory pathway is essentially composed of two pathways. The first is the lemniscal, characterized by quick transmission to thalamus and cortex, response to high rates of stimulation, and by rapid adaption. This pathway is associated with epicritic (well localized) pain, and is important for animal survival in signalling those stimuli which threaten its integrity. The second is the antero-lateral which is in part constituted by a high threshold afferent system and which responds to intense thermal, chemical and mechanical stimuli. This system informs the animal about the state of tissue injury.

Inflammatory pain involves high threshold polymodal nociceptors associated with C fibres (Burgess & Perl, 1973) and the threshold of these nociceptors is lowered by prostaglandins (Perl, 1976).

In this paper recent observations which help us to understand the mechanism of action of aspirin-like drugs, the analgesic effect of opiate antagonists and some of the biochemical events underlying inflammatory hyperalgesia will be reviewed.

Early experiments

It has been shown (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971) that the synthesis of prostaglandins is inhibited *in vitro* and *in vivo* by aspirin-like drugs. Willis (1969) has demonstrated a prostaglandin E_2-like substance in experimental inflammatory exudates in rats; this substance has since been shown to be present in man in contact dermatitis and in lesions produced by ultraviolet light (Greaves, Sondegaard & McDonald Gibson, 1970).

Intravenous prostaglandins cause headache (Bergström, Düner, von Euler, Pernow & Sjovale, 1959) and pain along the veins into which they are infused (Collier, Karim, Robinson & Somers, 1972). Intramuscular injections may also cause severe long-lasting pain (Karim, 1971). In mice prostaglandins are powerful inducers of writhing (Collier & Schneider, 1972). Solomon, Juhlin & Kirschenbaum (1968) and Michaelsson (1970) have reported that intradermally administered prostaglandins cause hyperalgesia.

In contrast to bradykinin, prostaglandins have failed to produce pain when instilled on to a blister base (Horton, 1963), and hyperalgesia has not been reported to accompany dermal vasodilatation (Crunkhorn & Willis, 1969).

Early research has often been directed towards identifying a pain mediator able to cause overt pain,

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and has failed to recognize the importance of hyperalgesia produced by prostaglandins (E_1, E_2 and $F_{2\alpha}$). This oversight may partly have been due to a lack of parallel assay of other putative inflammatory pain mediators.

Ferreira (1972) has shown that the intradermal administration of histamine, bradykinin, acetylcholine and prostaglandin E_1 produces overt pain of short duration, but only prostaglandin produces hyperalgesia, in some cases for several hours.

Subcutaneous infusions of histamine, bradykinin or prostaglandins (E_1, E_2 or $F_{2\alpha}$) do not produce overt pain. The addition of PGE_1 to bradykinin or to histamine is overtly painful. The addition of bradykinin to histamine is not. In areas in which prostaglandins have already produced hyperalgesia, subsequent infusions of bradykinin or histamine cause pain. These studies show that prostaglandins but not histamine or bradykinin are able to sensitize pain receptors to mechanical and chemical stimulation.

Although infusions of prostaglandin E_2 or $F_{2\alpha}$ produce no hyperalgesia at 30 min, they do so at 2 hours. This suggests that the continuous release of prostaglandin in small, perhaps not detectable amounts, will eventually induce hyperalgesia.

Only infusions containing prostaglandins and histamine produce pruritus. That prostaglandins potentiate histamine-induced itch has since been confirmed by Greaves & McDonald-Gibson (1973).

Various studies have confirmed and extended these observations. The pain-sensitizing action of prostaglandins (E_2 and E_1) to mechanical or chemical stimulation (generally bradykinin) has been observed in several experimental models, such as: (a) the reflex increase in blood pressure in dogs induced by bradykinin injected into the splenic artery (Ferreira, Moncada & Vane, 1973), superfused over the epicardium (Staszewska-Barczak, Ferreira & Vane, 1976) or given into the knee joint (Moncada, Ferreira & Vane, 1975); (b) the dog incapacitation test (Rosenthale, Dervinis, Kassarich & Singer, 1972); (c) the Randall-Selitto test in the rat paw (Willis & Cornelsen, 1973; Ferreira, Nakamura & Castro, 1978); (d) the rabbit ear preparation (Juan & Lembeck, 1974); and (e) the writhing test in mice (Collier & Schneider, 1972). These studies have received the support of more direct observations carried out using electrophysiological techniques (Chahl & Iggo, 1977; Perl, 1976; Handwerter, 1976).

From these experiments it can be concluded that aspirin-like drugs do not interfere directly with the sensitizing action of prostaglandins or with the direct effect of nociceptive stimuli such as bradykinin. When aspirin-like drugs block the nociception induced by bradykinin, this is secondary to the inhibition of the local release of prostaglandins caused by bradykinin (Ferreira *et al.*, 1975; Juan & Lembeck, 1976). The hyperalgesia induced by prostaglandins probably results from lowering the normal high threshold of polymodal nociceptors associated with C fibres (Perl, 1976). Injury releases prostaglandin-like substances which are responsible for the development of inflammatory hyperalgesia. In this context, non steroid antiinflammatory drugs which inhibit the synthesis of prostaglandins (cyclo-oxygenase inhibitors) should be referred to as antialgics (or antialgesics) because they prevent the induction of hyperalgesia. The term analgesic should be reserved for drugs acting on already established hyperalgesia or overt pain. Opiates are central analgesics. Peripheral analgesia is practically obtained only with local anaesthetics.

Central antialgic effect of aspirin-like drugs

Lim, Guzman, Rodgers, Goto, Braun, Dickerson & Engle (1964) using the cross perfused spleen technique, have demonstrated that aspirin-like drugs act peripherally at the pain chemoreceptors and that narcotic analgesics affect the central pathways for pain. All our previous experiments have supported this view. However, we have been puzzled by the fact that in rats a second injection of carrageenin into the contralateral paw, instead of producing less hyperalgesia (as occurs with the oedema) produces a hyperalgesia of equal intensity but with a much faster development. This may indicate that the first local inflammation evokes a central mechanism which facilitates the hyperalgesia induced by the second injection of carrageenin. We have investigated whether this facilitation could be caused by a release of a prostaglandin-like substance in the CNS evoked by the first inflammatory stimulus. In these experiments (Ferreira, Lorenzetti & Correa, 1978) rat paw hyperalgesia was measured using a modification of the Randall-Selitto test (1957). First, it was shown that administration of prostaglandin E_2 into the cerebral ventricles potentiated the hyperalgesic effect obtained by intraplantar administration. It was only with combined intracerebroventricular and intraplantar injections that the hyperalgesia caused by carrageenin administered into the paw was attained. Carrageenin-evoked hyperalgesia was diminished either by intracerebroventricular or intraplantar injections of a prostaglandin antagonist (SC-19220). The combined central and peripheral administration produced additional effects. Finally, it was shown that aspirin, indomethacin, paracetamol and phenacetin caused an antialgic effect on the carrageenin hyperalgesia test. There was a synergic antialgic effect between central and peripheral administration of these agents. These results suggest

that, in the rat, inflammatory hyperalgesia induced by carrageenin has two components resulting from prostaglandin release: a peripheral one due to a local sensitizing action and a central one, following the release of prostaglandins in the CNS which lowers the threshold of the central pain circuit. It is not known if this idea can be extended to other models of inflammation but it may be applicable to models in which fever due to prostaglandin release in the CNS occurs during the development of an inflammation.

Comparison of the hyperalgesic effect induced by prostaglandins and prostacyclin (PGI_2)

Prostaglandins of the E series cause long-lasting hyperalgesia. This may explain why in various pathological conditions such as rheumatoid arthritis or special types of headache there is a delay of several hours before the antialgic effect of cyclo-oxygenase inhibitors (such as non-steroidal antiinflammatory drugs) becomes apparent. However, in many clinical situations and experimental animal models, the antialgic effect of aspirin-like drugs is relatively rapid. Prostaglandin hyperalgesia in indomethacin (2 mg/kg) treated animals lasts over 5 h, as does as carrageenin-induced hyperalgesia. However, if indomethacin is given when carrageenin-induced hyperalgesia reaches a plateau there is rapid restoration of the pain threshold.

Indomethacin only partly affects established incapacity induced by endotoxin administration to the dog knee joint. When given before the challenge, it completely blocks the development of hyperalgesia.

These results suggest that in the rat, prostaglandins of the E series cannot account for hyperalgesia induced by carrageenin and that in dog they only contribute in part to the incapacitating effect.

An explanation for this discrepancy has recently been suggested (Ferreira, Nakamura & Castro, 1978). prostacyclin has been shown to be more potent than PGE_2 in both rat and the dog models. Furthermore hyperalgesia induced by prostacyclin is immediate and of shorter duration. Prostacyclin, like prostaglandins of the E series and thromboxane, is a product of the oxidation of arachidonic acid by cyclo-oxygenase. The synthesis of these compounds is blocked by aspirin-like drugs. The presence of 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin has been demonstrated in inflammatory exudates (Chang, Murota & Tsurufugi, 1976). The immediate and short lasting effect of prostacyclin has been confirmed by Higgs, Moncada & Vane (1978). Thus, the action of aspirin-like drugs in a hyperalgesic state may indicate that prostacyclin or prostaglandins of the E series are participating in that process.

Prostaglandin hyperalgesia as a mechanism related to cyclic AMP/Ca^{2+}

Interesting characteristics of prostaglandin E_2 hyperalgesia are firstly its long duration and secondly the long time before it reaches its plateau after a single dose (Ferreira *et al.*, 1978). In the rat paw or dog knee joint the maximum effect of small doses of prostaglandin E_2 is attained 2–3 h after its administration. This delayed and long-lasting hyperalgesic effect suggests a basic change in the metabolism of nociceptors. Recently, Eccles & McGeer (1979) have called attention to two different features of neurotransmission. In ionotropic transmission the mediator acts directly to open ionic gates, whereas in the metabotropic transmission the mediator acts indirectly by triggering a biochemical change at the postsynaptic membrane. In contrast to metabotropic transmission ionotropic possesses short latency and increases conductance at the postsynaptic membrane. This concept may apply to nociceptors related to inflammatory hyperalgesia. Considering the differences in their mode of action (Moncada, Ferreira & Vane, 1978) bradykinin and prostaglandin effects could be considered as ionotropic and metabotropic, respectively.

In order to examine the hypothesis that prostaglandin hyperalgesia is a metabotropic effect we have investigated the possibility of mimicking prostaglandin hyperalgesia by cyclic adenosine monophosphate (cyclic AMP) and by substances known to modulate its metabolism (Ferreira & Nakamura, 1979*a*).

In these experiments hyperalgesia was measured by our modification of the Randall-Sellito method (Ferreira, Lorenzetti & Correa, 1978). Rats were treated with indomethacin 2 mg/kg intraperitoneally for 30 min before starting the experimental session, in order to avoid the release of prostaglandins by the trauma of injection or by the injection of test substances.

The time course of hyperalgesia induced by intraplantar injection of prostaglandin E_2, dibutyryl cyclic AMP, isoprenaline, $BaCl_2$ and Ca^{2+} ionophore (A 23187) was similar. The plateau effect was attained within 2–3 h and remained up to the fourth or fifth hours. Prostaglandin E_2-induced hyperalgesia at doses in the nanogram range, whereas 500–1,000-fold greater quantities of other agents were necessary to cause an equivalent effect. All agents caused a dose-dependent effect and were potentiated by local administration of methyl-xanthines. Caffeine 10 μg and theophylline 20 μg had no analgesic effect on normal paws. Potentiation of the hyperalgesic effect of prostaglandin E_2 by methyl-xanthines could only be demonstrated in rats not pretreated with indomethacin, as this inverted the effect of the methyl-xanthines,

causing analgesia instead of enhancing hyperalgesia. Indomethacin treatment had no effect on other hyperalgesic stimuli. Adrenaline 100 μg was equipotent to isoprenaline and twice as potent as noradrenaline. Hyperalgesia induced by isoprenaline was abolished by propranolol which, in contrast, had no effect on prostaglandin E_2-induced hyperalgesia, thus indicating that the effect of the sympathomimetic is mediated by way of β-receptor.

These results support the hypothesis that rat paw hyperalgesia induced by prostaglandin is related to a cyclic AMP/Ca^{2+}-dependent process. We suggest that the interaction of prostaglandin or catecholamines with the receptor at the peripheral nociceptive terminations activates an adenyl cyclase associated with pharmacological receptors, causing an increase in the intracellular concentration of cyclic AMP. This assumption is based on the fact that local administration of dibutyryl cyclic AMP or of andeyl cyclase activators such as catecholamines and prostaglandins induced hyperalgesia. This local hyeralgesic effect of dibutyryl cyclic AMP may explain the observation that a single intravenous injection of this cyclic AMP derivative in man causes headaches, abdominal pain, myalgias and other types of pain (Levine, 1970). In several biological systems increased levels of cyclic AMP are associated with an increased Ca^{2+} concentration (Greengard, 1979). In our system substances which increase intracellular Ca^{2+} concentration ($BaCl_2$ and Ca^{2+} ionophore) induced hyperalgesia, whereas verapamil and lanthanum (known to block Ca^{2+} influx) were analgesics. A Ca^{2+} related mechanism may also be involved in CNS activity, as intraventricular administration of lanthanum causes antinociception (Iwamoto, Harris, Loh & Way, 1978). On the basis of our results it is difficult to know whether intracellular Ca^{2+} concentration regulates adenylate cyclase activity or *vice versa*. It seems that in our system methyl-xanthines potentiate hyperalgesia by a mechanism other than inhibition of phosphodiesterase, as dibutyryl cyclic AMP, a resistant cyclic AMP derivative (Sutherland, Robisom & Butcher, 1968), in the same way as PGE_2, isoprenaline, $BaCl_2$ and Ca^{2+} ionophore, was also potentiated. In our working hypothesis, intracellular concentrations of Ca^{2+} at nociceptors play a major role in controlling their threshold sensitivity, possibly modulating adenyl cyclase and thereby the induction of generator potentials.

Cyclic guanosine monophosphate (cyclic GMP) in several biological systems, has been shown to have the opposite effect to cyclic AMP (Goldberg, Haddox, Nicol, Glass, Sanford, Kuehl & Estensen, 1975). In our system, direct administration of cyclic GMP or substances which stimulate the synthesis of cyclic GMP (acetylcholine and carbachol) caused peripheral analgesia. The effects of acetylcholine or carbachol were associated with a muscarinic receptor, as atropine abolished the analgesia. It is interesting that the analgesic effect of cholinergic substances could only be demonstrated after treatment of rats with indomethacin. This may be due to a local release of prostaglandins, as it also occurs in isolated lungs after acetylcholine administration (Alabaster & Bakhle, 1976). The peripheral analgesic effect of cyclic GMP seems to occur at various levels in the CNS, as its central administration also causes antinociception (Conn, Conn & Taylor, 1978). We have shown that substances which lower intracellular Ca^{2+} concentration were analgesics but there is no experimental evidence indicating that this effect is related to an increased activity of cyclic GMP. The importance of the analgesic effect of cyclic GMP for development of a new class of peripheral analgesic agents is discussed later.

Peripheral action of morphine and its antagonists

The results of Ferri, Santagostino, Braya & Galatulas (1974) and Collier & Roay (1974) have suggested that morphine-induced central analgesia may result at least in part from an inhibition of activation of adenylate cyclase by prostaglandins. This prompted us to test whether prostaglandin hyperalgesia could be blocked by morphine.

It has been found that morphine and enkephalins have a peripheral analgesic effect (Ferreira & Nakamura, 1979*b*). Hyperalgesia due to intraplantar injection of PGE_2 is fully developed by about 3 h and remains constant for up to 6 hours. The intraplantar injection of morphine 10 μg given when hyperalgesia is already fully developed greatly reduces its intensity for 2.5 hours. Met-enkephalin has a similar effect but of shorter duration at the dose used (50 μg). Both substances cause hypoalgesia when injected in control saline treated paws. Morphine treatment of the contralateral paw does not affect the intensity of hyperalgesia induced by prostaglandin or the threshold of saline-treated paws. This result excludes a central component in the peripheral analgesia observed.

To characterize further this peripheral site of action of morphine, we have investigated the effects of naloxone, a 'pure' morphine antagonist. The results have shown unexpectedly that naloxone itself has an agonist morphine-like effect in reducing hyperalgesia. Furthermore, naloxone in equimolar concentration and given at the same time as morphine does not cause any antagonism of the analgesic effect but produces an additive effect greater than that attained by each substance given separately. Although naloxone is generally accepted

as a 'pure' antagonist it might have partial agonist activity at this peripheral site.

We have estimated the potency of opiates and enkephalins at this peripheral site, relative to a standard locally acting analgesic agent lidocaine. Calculated from linear regression the ID_{50} value for each substance was: morphine 14.3 nmol; nalorphine 17.8 nmol; naloxone 20.4 nmol; pentazocine 22.4 nmol; met-enkephaline 74.5 nmol; leu-enkephalin 126.6 nmol and lidocaine 1774 nmol. Two important points emerge firstly, lidocaine is about 100 times less potent than the opioid agonists and antagonists. Therefore, the observed effects cannot be simply explained by a local anaesthetic activity. Secondly, although enkephalins are rapidly inactivated by plasma, met-enkephaline was only four times less potent that morphine. The comparative low potency of leu-enkephalin is in accord with its potency in other systems.

Morphine and met-enkephalin has a clear peripheral analgesic effect on prostaglandin as well as on carrageenin hyperalgesia. Morphine also reduces the hyperalgesic effect of isoprenaline and $BaCl_2$ but has no effect on dibutyryl cyclic AMP hyperalgesia. This observation is in accord with our hypothesis that these analgesics act on a step preceding cyclic AMP generation in the process by which prostaglandins activate adenyl cyclase.

Relevance of the peripheral analgesic effect of morphine and of morphine antagonists

In order to compare the central and peripheral effects of these agents we have investigated their activity after administration into the cerebral ventricles (Ferreira & Nakamura, 1979*c*). The doses given intraventricularly were in the ID_{50} range calculated from the peripheral analgesic effect. The tests were carried out on groups of five rats and the results expressed as the mean values obtained 4.5 h after the induction of hyperalgesia by prostaglandins and 2 h after the intraventricular injection of the test drug. Control intraventricular injection of saline (10 μl) had no effect on the threshold response of normal (46±0.15 s) or of hyperalgesic paws (19.7±0.4 s). Morphine caused an intense analgesia in both control (8.8±4.3 s) and hyperalgesic (6.3±1.8 s) paws (compare with the former group). For naloxone the intensity of hyperalgesia was: 7.2±0.5 s for the control and 25.4±0.4 s for hyperalgesic paws. The values for nalorphine-treated rats were 8.7±0.6 s and 26.0±0.7 s for control and hyperalgesic paws, respectively. Intraventricular administration of pentazocine did not change the response of normal (0.3±0.1 s) or hyperalgesic paws (19.8±0.3 s). These results indicate that morphine antagonists, in contrast to morphine, are devoid of central analgesic effects. In fact, naloxone and nalorphine caused hyperalgesia.

In the next series of experiments (Ferreira & Nakamura, 1979c), we have looked for peripheral activity after systemic (intraperitoneal) injection of morphine and morphine antagonists in an attempt to evaluate the contribution of peripheral analgesia to the total analgesic effect. The intraperitoneal doses used have been shown to be effective in the original Randall-Selitto test but are generally ineffective in tests based upon reaction to thermal stimuli (Ward, Foxwell & Funderburk, 1965; Winter & Flataker, 1965). All agents cause analgesia 30 min after systemic administration. However, with nalorphine and naloxone the analgesic activity was short-lasting and after an hour was replaced by a hyperalgesic effect, manifest in both prostaglandin and saline-treated paws, and further increased at the second hour. This hyperalgesia is probably due to a central effect, as these agents have only analgesic activity when given by the intraplantar route. Because pentazocine was devoid of central effect in our test its systemic effect, which lasted over 2 h, is probably only due to a peripheral action.

Conclusions

The antialgic effect of aspirin-like drugs may be explained if prostaglandins are responsible for inflammatory hyperalgesia. But will this allow the improvement of this group of agents? It is thought that some side-effects of aspirin-like drugs also depend on their action upon gut or platelet cyclo-oxygenase. The differential sensitivity of tissue cyclo-oxygenase to non-steroid antiinflammatory drugs supports this possibility. Paracetamol and dipyrone, which are more potent on brain than on prostaglandin synthetase of other tissues (Flower & Vane, 1972; Dembiska-Kiec, Zmuda & Krupinska, 1976), have a more intense antialgic and antipyretic action relative to their effect upon oedema or erythema. The possibility of developing a potent antialgic or antiinflammatory agent with little selectivity for gastric cyclo-oxygenase remains open to investigation. A prostaglandin E_2 antagonist would only be effective in certain types of hyperalgesia. A prostacyclin antagonist, besides being an analgesic, would facilitate thrombus formation (Moncada, Higgs & Vane, 1977) and may cause gastric irritation (Whittle, 1976).

The discovery and synthesis of enkephalins provided the opportunity to look for endogenous substances with opiate-like analgesic activity. Unfortunately it has not been possible to dissociate their central analgesic effect from their dependence-producing properties.

Our results clearly indicate a new approach to the search for analgesics. The observed peripheral analgesic activity of morphine antagonists suggests the presence of a receptor different from that already postulated in the CNS and in the periphery (Knoll, Furst & Makleits, 1977; Martin, 1967). At peripheral nociceptors naloxone and nalorphine are agonists as potent as morphine, pentazocine or met-enkephalin (N-receptor) in contrast to their antagonist effect on central anagelsic morphine (M) receptors. It could be desirable to develop drugs which act exclusively at the N-receptors. We postulate that the N-receptors are associated with inhibition of an adenyl cyclase capable of being stimulated by prostaglandins and β-sympathomimetics.

The ideal peripheral analgesic should not be able to cross the blood-brain barrier (thus avoiding any central side-effect) but should have a strong peripheral action on inflammatory hyperalgesia. Many of the presently used assays measure mainly analgesia at a central site, selecting for pain-relieving properties as well as for addiction liability (Lasagna, 1968; Ward *et al.*, 1965). However, the ideal analgesic should show high activity in the peripheral assays and no effect in the central assays. Such a compound might be one excluded from the CNS by its physicochemical characteristics, and should be inactive in the tail flick test. We recently described a prototype of such an agent (Ferreira & Nakamura, 1979*c*). BW 180c is an enkephalin analogue displaying increased resistance to hydrolysis, and which does not cross the blood-brain barrier (Beddell, Follenfant, Lowe, Ubatuba, Wilkinson & Miller, 1977). BW 180c has a strong local analgesic effect and, when given intraperiotonially, antagonizes both prostaglandin E_2 and carrageenin-induced hyperalgesia. We expect a similar effect in morphine antagonists or opiate analogues which do not cross the blood-brain barrier. Other useful agents may be long-lasting cyclic GMP derivatives. This substance is thought not to cross the blood-brain barrier and to have an analgesic effect when administered systemically.

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Discussion

DR BRUNE asked Dr Ferreira to give some details about the administration of drugs or prostaglandins to the brain and paw, for example, concerning the solvents, and treatment of the controls.

PROFESSOR FERREIRA replied that a colleague had developed a technique of injecting 10 μl into the cerebral ventricles over a 5-min period (Correa, F.M.A. & Graeff, F.G., *Neuropharmacology,* **13**, 65, 1974). The animal is anaesthetized, the injection given, and within 10 min the animal is completely awake again. For the paw saline is usually the solvent, but with some substances, according to the concentration required, ethanol has to be used (as the solvent). The control receives the solvent only by the same route of administration.

DR SUNSHINE asked whether prostaglandin mechanisms could explain the differences in ceiling analgesic effects between aspirin and newer drugs.

DR FERREIRA suggested it could be due to distribution differences. Alternatively he emphasized that the enzymes referred to as "prostaglandin synthetase" are not homogeneous, but show differential sensitivity. For example, the brain enzyme is very sensitive to dipyrone or paracetamol, but vascular enzymes are less sensitive. He added that it is known that prostaglandins cause hyperalgesia. But there may be other mediators which are released in inflammation, and some drugs may also have an effect upon these. That was the basis for his search for other mediators.

DR SUNSHINE asked if the addition of aspirin to the newer anti-inflammatory drugs would produce a summation of effects.

DR FERREIRA preferred not to theorize, but Dr Higgs said he would not expect a greater activity.

PROFESSOR SZCZEKLIK noted that prostacyclin was introduced last year for the treatment of peripheral artery disease. It was given intra-arterially over a period of 3 days. Some patients experienced minor pain in the leg or headache. It was his impression that the pain decreased as the (infusion) proceeded, in other words, there was less pain after 3 days of such an infusion than during the first few hours. As both Dr Higgs and Dr Ferreira showed that prostacyclin had some hyperalgesic effect, he wanted to know whether animal studies or studies in man suggested a desensitization to the hyperalgesic effect?

DR FERREIRA did not know of any.

DR HIGGS wondered to what extent the pain and headache following prostacyclin infusion could be related to the vasodilator activity of prostacyclin? Was it possible that a systemic compensating mechanism to such vasodilatation existed?

DR VON GRAFFENRIED asked Dr Ferreira if he had implied that morphine had a direct peripheral effect, which was not antagonized by naloxone.

DR FERREIRA replied that he had, and found difficulty in understanding the results of his experiments. He added that there were several situations in which naloxone was unable to block morphine.

PROFESSOR BEAVER asked whether Dr Ferreira envisaged the central prostaglandin component in hyperalgesia as being associated with prostaglandin released within the CNS as a mediator of neurotransmission, or something that leaks into the CNS as a result of tissue destruction and inflammation in the periphery which then has a CNS effect?

DR FERREIRA replied that fever accompanying inflammation could generate endogenous pyrogen, which stimulated some cell in the CNS to generate prostaglandin. The inflammatory stimulus could, however, have a local and central effect.

PROFESSOR BEAVER suggested that higher lipid solubility could enable agents to penetrate in significant quantities into parts of the CNS that some of the more conventional antipyretic analgesics do