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EFFECTS OF INTRACEREBROVENTRICULAR FLOCTAFENINE AND INDOMETHACIN ON BODY TEMPERATURE IN FEBRILE RABBITS

HELEN LABURN, D. MITCHELL & J. STEPHEN

Department of Physiology, University of the Witwatersrand Medical School, Johannesburg 2001, South Africa

1 We injected the potent prostaglandin synthesis inhibitors, floctafenine and indomethacin, intravenously and intracerebroventricularly in rabbits made febrile by intravenous injection of leucocyte pyrogen.

2 Floctafenine (75 μmol) injected intravenously failed to affect the fever, whereas indomethacin (15 μmol) markedly reduced the fever.

3 When injected into the cerebral ventricles, floctafenine was feebly antipyretic, and then only at a dose ten times the antipyretic dose of indomethacin.

4 Our results support the suggestion that floctafenine has no antipyretic action when administered peripherally. However, its lack of antipyretic effect cannot be explained solely on the grounds that it fails to cross the blood-brain barrier.

Introduction

The analgesic, anti-inflammatory, and antipyretic properties of the aspirin-like drugs are usually attributed to their ability to inhibit the enzymes which convert arachidonic acid and linolenic acid into prostaglandins and related products (Vane, 1971). Floctafenine (Idarac) is a potent inhibitor of prostaglandin synthesis, and is analgesic and anti-inflammatory (Peterfalvi, Deraedt, Benzoni, Chifflot & Fournex, 1975). Although no formal tests of its antipyretic activity have been described in the literature, floctafenine is reputed to have no antipyretic nor other central nervous system effects. Antipyresis occurs as a result of drug action on neurones of the hypothalamus (Bernheim, Block & Atkins, 1979) whereas the analgesic and anti-inflammatory actions of the aspirin-like drugs are predominantly peripheral.

We have investigated the effects of floctafenine, injected both intravenously and intrathecally, on the body temperature of febrile rabbits and have compared the action of floctafenine with that of indomethacin. Floctafenine and indomethacin are inhibitors of prostaglandin synthesis of similar potency (Deraedt, Jouquey, Benzoni & Peterfalvi, 1976).

Some of the results have been reported briefly to the Physiological Society of Southern Africa.

Methods

The experiments were carried out in adult male and female New Zealand White rabbits, weighing between 2.5 kg and 3.0 kg, at a neutral ambient temperature. The rabbits were conscious and restrained in conventional stocks. Fever was produced by intravenous injection of 3 ml of homologous leucocyte pyrogen made from whole rabbit blood (Borsook, Laburn & Mitchell, 1978).

Peripheral drug administration

Floctafenine and indomethacin, or equal volumes of their solvents, were injected at the same time as the leucocyte pyrogen into a marginal ear vein. Floctafenine was injected in a dose of 75 μmol suspended in 3 ml ethanol. Indomethacin was injected in a dose of 15 μmol in 3 ml ethanol. Control injections consisted of 3 ml ethanol. Each procedure was carried out on five rabbits.

Central drug administration

Floctafenine and indomethacin, or their solvents, were microinjected into the lateral cerebral ventricles of rabbits at the same time as the intravenous injection of leucocyte pyrogen. Access to the ventricles was obtained by fixing a metal headplate to the skull of each rabbit at least one week before the experiment. The stereotaxic positioning of the headplate and the procedure for making microinjections into the lateral ventricles was a modification of the technique of Monnier & Gangloff (1961) and has been described previously (Rosendorff & Mooney, 1971).

Floctafenine and indomethacin were each injected

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in doses of 28 nmol, 280 nmol and 2800 nmol. Floctafenine was dissolved in dimethyl sulphoxide. Indomethacin was dissolved in ethanol. Control injections consisted of either dimethyl sulphoxide or ethanol alone. Test and control solutions were injected in a total volume of 10 μl and were flushed through the injection cannulae with 30 μl of sterile water. Six rabbits were used for each test procedure and five rabbits for each control procedure.

Temperature measurements

Rectal temperatures were measured by means of indwelling thermistor probes inserted approximately 100 mm into the rectum. The outputs of the thermistors were recorded using a bridge circuit (YSI 47A telethermometer) and chart recorder (Kipp BD5). The febrile response was calculated in terms of the change in rectal temperature from that prevailing at the time of injection. All data were subjected to Student's t test and values of $P < 0.05$ were considered statistically significant.

Drugs

Indomethacin was obtained from Sigma, and stored at 0°C until used. Floctafenine (2,3 dihydroxypropyl *N*-(8-trifluoromethyl 4-quinolyl) anthranilate) was obtained from Roussel Laboratories.

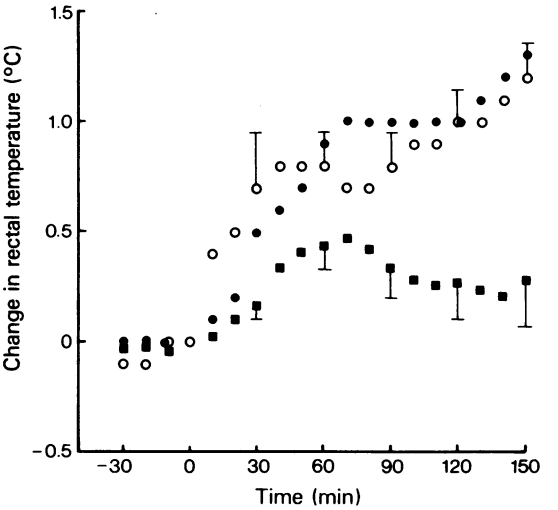

Figure 1 Mean changes in rectal temperature from those prevailing at the time of injection (time zero) in the five rabbits given 3 ml of leucocyte pyrogen intravenously and simultaneously intravenous ethanol 3 ml (○), intravenous floctafenine 75 μmol in 3 ml ethanol (●) or intravenous indomethacin 15 μmol in 3 ml ethanol (■). Changes in rectal temperature following floctafenine injection were never significantly different from those following ethanol alone ($P > 0.05$, t test). Following indomethacin injection, mean change in rectal temperature was not significantly different from zero ($P > 0.05$, t test) from 80 min. Error bars denote one standard error.

Results

Intravenous injections

Figure 1 shows the effects of intravenous injection of floctafenine and indomethacin, and their vehicle ethanol, on the fever produced by the simultaneous intravenous injection of leucocyte pyrogen. Rectal temperatures following the injection of 75 μmol of floctafenine with the leucocyte pyrogen were never significantly different from the febrile rectal temperatures which followed the injection of leucocyte pyrogen and ethanol. However, when 15 μmol of indomethacin was injected with the leucocyte pyrogen, the rectal temperature rise was suppressed 80 min after the injection, and for the remainder of the experiment, the average rise in rectal temperature following indomethacin injection was not significantly different from zero ($P > 0.05$, t test).

Intracerebroventricular injections

Figure 2 shows the effects on rectal temperature of the intracerebroventricular microinjection of floctafenine simultaneous with intravenous injection of leucocyte pyrogen. Control injections consisted of intravenous leucocyte pyrogen together with intracerebroventricular microinjection of the drug vehicle, dimethyl sulphoxide. Rectal temperatures following injection of 28 nmol and 280 nmol of floctafenine were not significantly different from those following injection of dimethyl sulphoxide. The highest dose of floctafenine, 2800 nmol, significantly depressed the fever ($P < 0.05$, t test) but only for the 30 min between 60 min and 90 min after injection. Thereafter, rectal temperatures at even this high dose of floctafenine were not significantly different from the rectal temperatures in the control fevers.

In contrast, as Figure 3 shows, intracerebroventricular microinjection of indomethacin in doses of 280 nmol and 2800 nmol significantly attenuated the fever induced by leucocyte pyrogen injection. In the case of the 280 nmol dose, rectal temperatures were significantly lower ($P < 0.05$, t test) from 110 min after injection. In the case of the 2800 nmol dose, rectal temperatures were significantly depressed ($P < 0.01$) from 80 min after injection. The antipyretic action of indomethacin does not depend on the alcohol vehicle. We have previously shown intraventricular injections of indomethacin to be antipyretic when the vehicle is

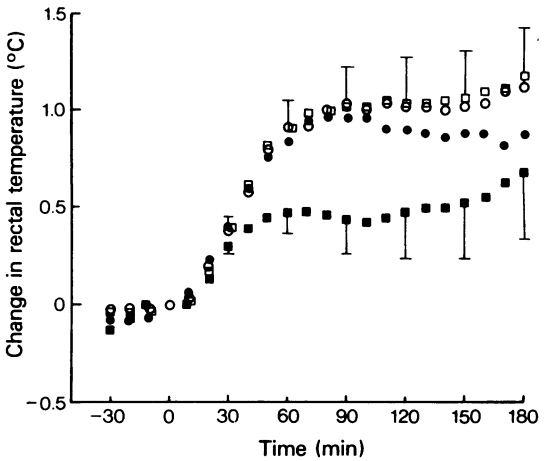

Figure 2 Mean changes in rectal temperature from those prevailing at injection (time zero) in five or six rabbits given 3 ml of leucocyte pyrogen intravenously and simultaneously intracerebroventricular dimethyl sulphoxide 10 µl (○), floctafenine 28 nmol in 10 µl dimethyl sulphoxide (●), floctafenine 280 nmol in 10 µl dimethyl sulphoxide (□) and floctafenine 2800 nmol in 10 µl dimethyl sulphoxide (■). Rectal temperatures following 2800 nmol floctafenine were significantly different ($P < 0.05$, t test) from those following dimethyl sulphoxide 10 µl (○), floctafenine 28 nmol in 10 µl dimethyl sulphoxide (●), floctafenine 280 nmol in 10 µl Error bars denote one standard error.

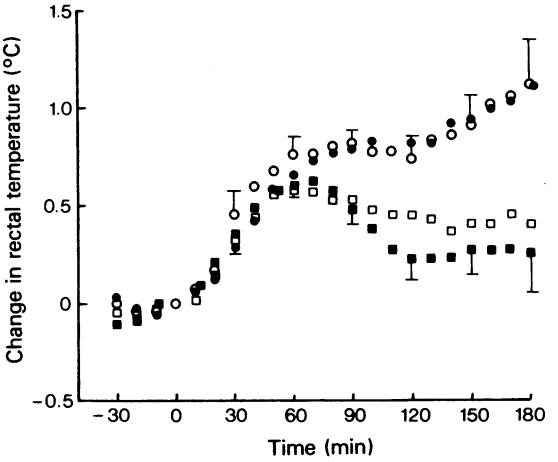

Figure 3 Mean changes in rectal temperature from those prevailing at injection (time zero) in five or six rabbits given 3 ml of leucocyte pyrogen intravenously and simultaneously intracerebroventricular ethanol 10 µl (○), indomethacin 28 nmol in 10 µl ethanol (●), indomethacin 280 nmol in 10 µl ethanol (□) and indomethacin 2800 nmol in 10 µl ethanol (■). Rectal temperatures following 280 nmol indomethacin were significantly different ($P < 0.05$, t test) from those following ethanol alone at 110 min and afterwards; following 2800 nmol they were highly significantly different ($P < 0.01$) at 80 min and afterwards. Error bars denote one standard error.

dimethyl sulphoxide (Laburn, Mitchell & Rosendorff, 1977).

Discussion

The aspirin-like drugs are thought to exert their antipyretic, anti-inflammatory and analgesic effects by inhibiting the cyclo-oxygenase enzymes which synthesize the prostaglandin endoperoxides from their parent fatty acids, arachidonic acid and linolenic acid (Vane, 1971; Vane & Ferreira, 1979; Clark, 1979). The endoperoxides are the precursors of both the prostaglandins and the thromboxanes. Floctafenine is an anthranilic acid derivative with aspirin-like properties; it is a potent inhibitor of prostaglandin synthesis, has anti-inflammatory and analgesic effects, but anomalously, is reported to have no antipyretic action (Peterfalvi *et al.*, 1975).

We have now investigated quantitatively the possible antipyretic action of floctafenine, using experimental leucocyte pyrogen fever in rabbits as a model. The model is an appropriate one for testing antipyretic drugs; intravenous injections of homologous leucocyte pyrogen in rabbits consistently produce fevers of short latency and of at least 3 h duration (Borsook *et al.*, 1978).

Floctafenine injected intravenously has no detectable antipyretic action, even at a dose as large as 75 µmol, or 25 to 30 µmol/kg. The oral therapeutic dose of floctafenine in man is 20 to 40 µmol/kg per day. Indomethacin, on the other hand, was significantly antipyretic at an intravenous dose of 15 µmol.

The analgesic and anti-inflammatory actions of the aspirin-like drugs are predominantly, if not entirely, peripheral, whereas the antipyretic actions are entirely central; the site of action of the antipyretic drugs is the hypothalamus (Bernheim *et al.*, 1979). One possible explanation for the failure of intravenous floctafenine to produce antipyresis is therefore that the drug fails to cross the blood-brain barrier, which would also account for the absence of any other central nervous system effects (Peterfalvi *et al.*, 1975).

If the reason that floctafenine is not antipyretic is that it cannot gain access to hypothalamic neurones from the intravascular space, then the drug should be antipyretic on intracerebral injection. We have injected floctafenine directly into the lateral ventricles of rabbits made febrile with intravenous leucocyte pyrogen. On intracerebroventricular injection, flocta-

fenine was antipyretic, but only feebly so, and then only at a dose ten times higher than the dose at which intracerebroventricular indomethacin was markedly antipyretic.

Previous evidence has shown that the antipyretic potency of the aspirin-like drugs correlates well with their ability to inhibit prostaglandin synthesis (Ziel & Krupp, 1974). *In vitro*, floctafenine inhibits the synthesis of prostaglandin by guinea-pig lung tissue as potently as does indomethacin, but inhibits synthesis by rat adipose tissue somewhat less potently than indomethacin; *in vivo*, floctafenine is a much more potent inhibitor than indomethacin of the prostaglandin synthesis which occurs in mice in response to intraperitoneal injection of acetic acid (Deraedt *et al.*, 1976). It is therefore indeed surprising that floctafenine and indomethacin, injected directly into the cerebrospinal fluid, are not equipotently antipyretic.

We know of no other aspirin-like drug having anti-inflammatory and analgesic properties but not having antipyretic properties. However, the aspirin-like drug, paracetamol, is an effective antipyretic but is reported to have no anti-inflammatory action (Flower & Vane, 1974). Flower and Vane suggested that the dissociation of antipyretic and anti-inflammatory properties in the case of paracetamol arises from heterogeneity in the cyclo-oxygenase enzymes. It may well be the case that cyclo-oxygenases are a family of isoenzymes, with different isoenzymes exhibiting different drug susceptibilities. Floctafenine could then be a potent inhibitor of peripheral cyclo-oxygenase isoenzymes but not of central isoenzymes, a property with considerable therapeutic benefit.

It remains possible that floctafenine indeed inhibits the central enzymes responsible for the breakdown of arachidonic acid, but that such inhibition has no effect on the development of fever. It has not been proven that arachidonic acid (or linolenic acid) are essential intermediates in the biochemical genesis of fever; prostaglandin synthesis, at least, does not appear to be essential (Cranston, Duff, Hellon, Mitchell & Townsend, 1976; Hellon, Cranston, Townsend, Mitchell, Dawson & Duff, 1980). The aspirin-like drugs have actions other than that of inhibiting cyclo-oxygenase enzymes (Cantor & Hampton, 1978; Splawinski, Wojtaszek & Swies, 1979). Whether such actions could account for their antipyretic properties is not known.

In conclusion, our results support the hypothesis that the prostaglandin synthesis inhibitor floctafenine has no antipyretic action when administered peripherally. We suggest, however, that the lack of antipyretic action cannot be attributed solely to exclusion of the drug by the blood-brain barrier. Floctafenine may be inactive against central cyclo-oxygenase isoenzymes.

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