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Prostaglandins in the Brain of Rats Given, Acutely, and Chronically, a Hyperthermic Dose of Met-Enkephalin

G. M. Scoto, C. Spadaro, S. Spampinato, R. Arrigo-Reina, and S. Ferri

Institute of Pharmacology and Pharmacognosy, University of Catania, Italy

Abstract. An enhanced prostaglandinlike activity is shown in homogenates of brain from rats treated intracerebroventricularly with 100 µg of met-enkephalin. The increase is significantly reduced by naloxone pretreatment. A relationship is proposed between generation of prostaglandins in the brain following met-enkephalin administration and hyperthermic effect of the opiatelike factor in the rat. Normalization of prostaglandinlike activity following chronic administration of met-enkephalin in the rat may also account for the development of tolerance to its thermic effect.

Key words. Met-enkephalin – Brain – Prostaglandins – Rat – Temperature

Morphine and other opiates acutely administered elicit a complex of stimulant and depressant effects, depending on species, dose, site of administration, and time of observation. Hyperthermia, locomotor activity, and vomiting may, for instance, be considered stimulant effects, whereas analgesia, hypothermia, and cough inhibition are some of the depressant effects. As far as the effects on temperature are concerned, in the rat, many reports demonstrate the dual response to morphine depending on the dose, low doses being hyperthermic and high ones hypothermic both after i. p. and i. c. v. administration (Lotti et al., 1965; Oka et al., 1971; Cox et al., 1976; Kaakkola and Ahtee, 1977).

A similar dual effect on temperature is displayed by the naturally occurring pentapeptide met-enkephalin, which confirms its morphinomimetic activity in this respect (Ferri et al., 1978). Moreover, evidence exists of tolerance and cross tolerance between met-enkephalin and the narcotic, since the opioid thermic effects are no longer evident in morphine-tolerant rats (Ferri et al., 1978).

In investigating stimulant effects of morphine, Collier et al., (1974a) found that morphine stimulated in vitro prostaglandin biosynthesis in rabbit brain homogenates. This stimulation might explain morphine-induced hyperthermia after i. c. v. injection, provided that sufficient concentrations of the narcotic may reach the hypothalamic thermostats and stimulate Prostaglandins (PGs), production. It is well known, in fact, that PGs are consistently hyperthermic when injected intrahypothalamically (Cammock et al., 1976). The present study was undertaken to investigate whether changes in PG synthetase activity occur in the brain of rats given an acutely and chronically hyperthermic dose of met-enkephalin, thus trying to correlate its effect with endogenous PG production in this species.

Materials and Methods

Experiments wer performed on male Sprague Dawley rats weighing 150–160 g and fed with a standard diet and water ad lib. The environmental conditions were standardized (22° C ± 2; 12 h artificial lighting per day). The rats were randomized into groups of 10 animals each.

The first group of rats received i.c.v. 100 µg of met-enkephalin (Calbiochem) dissolved in sterile saline in a volume of 10 µl. This dose was shown to be hyperthermic in the rat (Ferri et al., 1978). The second group of rats was treated, 30 min before met-enkephalin, with naloxone (HCl, Endo) 3 mg/kg/s.c. A third group of rats received, by i.c.v. injection, met-enkephalin 100 µg daily for 8 days. Control animals received saline only. For the central administration of met-enkephalin a cannula was implanted, as previously described (Ferri et al., 1974), in the right ventricle of the rat brain, and 5 days elapsed before experimentation. The rats were killed 30 min after the last administration (maximum hyperthermic effect of met-enkephalin) and their brains quickly removed and homogenized in 4 vol of ice-cold 0.06 M Bücher medium pH 7.4 with a Potter Elvehjem tissue grinder. The homogenates were centrifuged for 10 min at 900 g. To 2 ml of the supernatant 20 µg Na arachidonate (Sigma Chemical Co.) were added. After incubation for 30 min at 37° C, the tubes were immerged in boiling water for 1 min to terminate the reaction. After filtration and acidification at pH 3 with HCl 0.1 N, PGs were extracted with ethylacetate. The extract was dissolved in Tyrode's

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Table 1. Production of prostaglandin E-like material by brains of rats 30 min after intracerebroventricular administration of met-enkephalin

Treatment (10 animals/group)	PGE-like material (ng/g wet wt ± SEM)		
	Unincubated samples (0-time)	Samples incubated 30 min with Na arachidonate	Net production
Controls	32.10 ± 3.19	70.52 ± 3.49	38.42 ± 3.80
Met (100 µg i.c.v.)	63.60 ± 3.33*	160.71 ± 4.05*	97.11 ± 5.19*
Met (100 µg i.c.v./8 days)	37.76 ± 2.44	71.71 ± 2.46	33.95 ± 2.45
Nx (3 mg/kg/s.c.)	34.80 ± 3.40	71.03 ± 2.82	36.23 ± 2.24
Nx (3 mg/kg/s.c.) + Met (100 µg i.c.v.)	43.14 ± 2.81**	102.34 ± 4.22**	59.20 ± 5.75**

Met met-enkephalin-treated rats; *NX* naloxone-treated rats; *controls* saline-treated rats
* $P < 0.05$ vs. controls
** $P < 0.05$ vs. Met acutely administered

solution and aliquots of this solution were assayed for total PGs against reference PGE_1 (Upjohn) on rat stomach fundus strip (Gilmore et al., 1968) suspended in Tyrode's solution at 37° C gassed with 5% CO_2 in O_2, which contained a mixture of antagonists to make the reaction of the assayed tissue more specific for PG: atropine, mepyramine, and methysergide (200 ng/ml), and phenoxybenzamine and propranolol (1 µg/ml). In some experiments, no Na arachidonate was added to samples of supernatant from brain homogenates of different treatment groups, which were immediately boiled (0-time samples). Net production of PGs was calculated by subtracting the amount of PGs in these samples from those found after incubation in samples with added arachidonate. All data were analyzed with Student's *t*-test.

Results

An enhanced prostaglandinlike activity is shown in homogenates of brains from rats treated i.c.v. with met-enkephalin, 100 µg, in comparison with that found in the brain of saline-treated animals (Table 1). This increase is significantly ($P < 0.05$) reduced by naloxone 3 mg/kg (which is by itself ineffective) administered s. c. 30 min before met-enkephalin. Table 1 also indicates that after an 8-day treatment of rats with the pentapeptide, PG-like activity is similar to that of controls.

Discussion

Previous results of Collier et al. (1974a), who showed that morphine stimulates in-vitro prostaglandin production in rabbit brain homogenates, are thus reproduced when the opioid met-enkephalin is injected directly into the rat brain. This effect is mediated via specific opiate receptors since it is greatly reduced by the specific opiate blocker naloxone. Higher doses of naloxone should probably be used to obtain a complete prevention of met-enkephalin-induced effect, since it seems that endogenous ligands of opiate receptors are more resistant to antagonism by naloxone than narcotic analgesics (Kosterlitz and Hughes, 1975).

The increase in PG activity should be attributed, in very large part, to increased PG biosynthesis and not to prevention of their enzymatic breakdown, as can be deducted from the comparison of prostaglandinlike activity exhibited by unincubated samples (indicating the contribution to biosynthesis of endogenous substrate present in the brain) and that found in samples incubated with the precursor Na arachidonate. Moreover, it has been shown that narcotic analgesics do not inhibit prostaglandin dehydrogenase nor cause consistent reduction of the labelled PGE_2 or $PGF_{2\alpha}$ in vitro (Collier et al., 1974b).

Rat brain synthesizes small amounts of prostaglandins, predominantly $PGF_{2\alpha}$ (Folco et al., 1976; Nicosia and Galli, 1976; Wolfe et al., 1976). Biosynthetic capacity in vivo is much less than that found in vitro as soon as brain tissue is homogenized or sliced, but biosynthesis increases consistently under pharmacologic and pathologic conditions, thus more closely reflecting the in-vitro situation (Wolfe et al., 1976). On the other hand, prostaglandin $F_{2\alpha}$, as well as PGE_1 and PGE_2, cause hyperthermia when injected in the lateral ventricle of the rat (Poddubiuk and Kleinrok, 1976). Therefore, a relationship should be admitted between the generation of PGs in the brain after i.c.v. administration of met-enkephalin and the hyperthermic effect of the pentapeptide in the rat.

Some perplexities arise in attributing a "physiologic" role to met-enkephalin on temperature regulation because no clear evidence exists of an action of naloxone, per se, on basal temperature, as previously shown by Goldstein and Howery (1975) and by Ferri et al. (1978). The described effects should therefore be considered more likely of "pharmacologic" nature.

Normalization of prostaglandinlike activity following chronic administration of met-enkephalin in the rat might also account for the development of tolerance to its thermic effect in the same species (Ferri et al., 1978). Future work should ascertain (1) whether any correla-

tion exists between brain prostaglandin activity and hypothermia induced by high doses of met-enkephalin and (2) whether met-enkephalin activates PG biosynthesis directly or indirectly through neuromediators, such as biogenic amines, known to be involved both in PG synthetase activity and in temperature regulation. Dopamine, seems, in this respect, particularly interesting; not only is it one of the most effective activators of prostaglandin synthesis in the rat brain, at least in vitro (Wolfe et al., 1976), but it also induces changes, dose- and ambient-dependent, in rat temperature (Cox and Lee, 1977).

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