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Concerning the Specificity of the Hypothalamic Opiate Receptor Responsible for Food Intake in the Rat

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TEPPERMAN, F. S. AND M. HIRST. *Concerning the specificity of the hypothalamic opiate receptor responsible for food intake in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1141–1144, 1982.—Direct application of small quantities of morphine (a μ-opiate receptor agonist) to the ventromedial hypothalamus (VMH) of rats can induce a stimulated food intake. Because this effect is only partly reduced by the opiate antagonist naloxone, given into the VMH, various other studies were undertaken to examine characteristics of the receptors at this site. The opiate agonist levorphanol but not its stereoisomer dextrorphan effectively increased feeding. Codeine, a weak opiate ligand, was also ineffective as were the κ-opioid agonist ketocyclazocine and the σ-opioid agonist phencyclidine. The hyperthermia which accompanies peripheral and central injections of morphine was not observed after hypothalamic application of a quantity of leverphanol sufficient to stimulate feeding. This leads us to propose that the opioid receptors in the VMH are: (1) stereoselective; (2) responsive to μ-, but not κ- or σ-agonists; and (3) different from the receptors that elicit hyperthermia.

Opiate receptors Feeding Hyperthermia Ventromedial hypothalamus Opiates Morphine

ALTHOUGH the physiological functions of brain opiate systems have not as yet been established, there is evidence that they may participate in feeding behaviour and thermoregulation. Both peripheral and central injections of various opiate ligands are able to alter ingestive patterns in rats [1, 6, 7, 18, 20, 24, 28]. Depending on dose and experimental conditions, peripherally applied opiate agonists can cause hyperthermia or hypothermia in rats [2, 16, 24, 26, 28]. Narcotic antagonists applied alone have less evident actions [5, 14, 16, 23]. The effects produced are presumed to be associated with stereoselective opiate receptors since subcutaneous injections of the L(−)-isomer levorphanol elevated daytime feeding and core temperature in non-deprived rats, whereas the non-analgesic D(+)-stereosiomer, dextrorphan, was without effect [27]. Although temperature influences were not recorded, Sanger, *et al.* [22] found that two benzomorphan compounds (Mr 1452 and Mr 2266) reduced food intake in deprived rats when administered subcutaneously, while their (+) isomers did not. However, these stereospecific effects on feeding have not been verified by injections of isomers into specific brain sites.

In previous work, we have shown that low doses of morphine injected into the ventromedial hypothalamus (VMH) of rats can promote feeding and a simultaneous increase in core temperature. Both effects were antagonized effectively by subcutaneous injections of the opiate antagonist naloxone, but were less effectively reduced by VMH application of this drug [24]. While this lack of apparent antagonist specificity was considered to reflect the probability of a rapid diffusion of naloxone away from the injection site it could also have occurred if naloxone had a low affinity for the receptors that interact with morphine. This latter consideration is not without precedent, since naloxone has been found previously to be relatively ineffective in reducing the effectiveness of morphine in some behavioural paradigms [3,29].

Accordingly, we have examined the specificity of the receptor which influences food intake. Measurements of core temperature were determined concurrently to see if changes in temperature always paralleled alterations in food intake. To this end we administered several drugs into the VMH: levorphanol and dextrorphan were given to assess whether or not this receptor has specific stereochemical requirements; codeine, which is metabolized by hepatic biotransformation to morphine but has stimulant properties at central sites [10, 11, 13], was employed as a control for non-selective activities; and ketocyclazocine and phencyclidine were applied because they are able to interact with forms of opiate receptors other than the μ(morphine) receptor [9, 15, 17].

METHOD

Male Sprague-Dawley rats (260–285 g) were housed individually at 22°C ambient temperature and maintained with ad lib food pellets (Purina) and water on a 12-hr light-dark cycle, with light onset at 9:00. A stainless steel guide cannula (23 gauge) which extended towards the right ventromedial hypothalamus (VMH) was implanted stereotaxically into each animal while anaesthetized (Equithesin, 3.5 mg/kg body wt.). The stereotaxic coordinates used were measured from bregma and followed the atlas of Pelligrino, Pelligrino and

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Cushman [19]: AP=+0.4, L=−0.5 and depth from dura=−8.3. Except during injections when a 30-gauge injection cannula was inserted to a depth 0.5 mm below the guide cannula, a stainless steel pin was left in the cannula. Animals were allowed at least seven days to recover, during which they were handled and sham-injected to minimize the influences of these manipulations on the observed experimental responses.

On experimental days, animals were allowed free access to food pellets and water, and were handled periodically until they received VMH injections (between 12:15 and 12:45 hr). The injections were always given in 0.5 μl sterile, pyrogen-free saline. Just prior to injection, and at hourly intervals for three hours thereafter core temperatures were monitored by rectal probe (Yellow Springs Telethermometer). While the rats remained in their cages, the probe was inserted to a depth of 6 cm and left for 20–30 sec until the reading was stable. To assess food intake, a pre-weighed quantity of food pellets was presented to the animals following injection. The remaining pellets plus any spillage were weighed and replaced hourly for the 3-hour period.

Cannulae placements were always verified histologically. Results were analyzed for significance by the Randomized Block Analysis of Variance. Where significance was present, the Studentized range test [34] was used to assess significance between groups.

In Experiment 1, seven rats were given VMH injections of levorphanol tartrate monohydrate, 1.8 and 5.3 nmole (0.75 and 2.25 μg: Hoffman la Roche); dextrorphan tartrate monohydrate, 5.3 nmole (2.25 μg: Hoffman la Roche); or codeine phosphate, 5.3 nmole (2.24 μg: BDH). Injections were given according to a Latin Square design.

In Experiment 2, seven rats were treated with saline, or 5.3 nmoles of morphine sulfate (2.01 μg: May and Baker); ketocyclazocine HCl (1.70 μg: Sterling-Winthrop); or phencyclidine HCl (1.48 μg: Health and Welfare, Canada), again according to a Latin Square schedule.

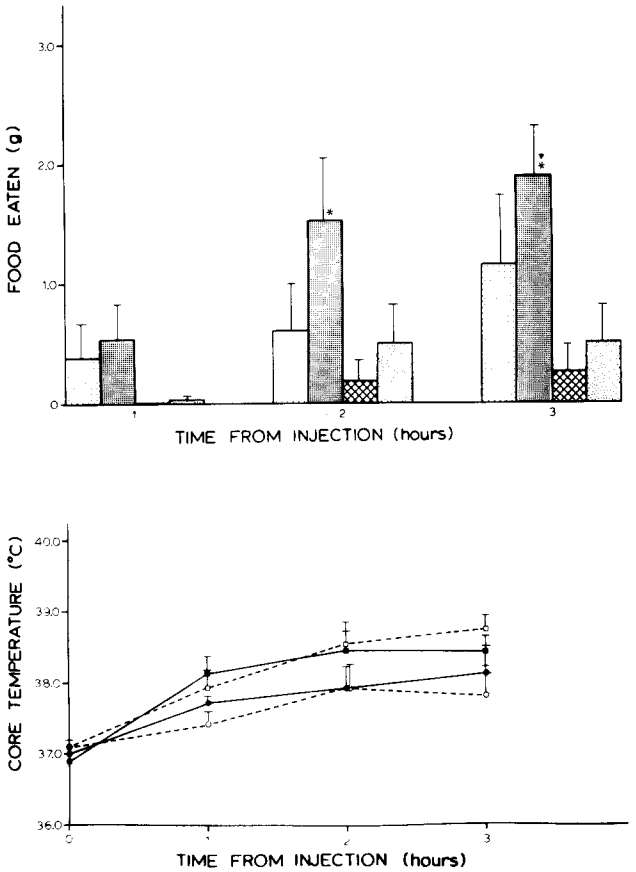

FIG. 1. Cumulative food intake and core temperature of rats (n=6) over 3 hours following administration into the ventromedial hypothalamus of: □, ●−● levorphanol (1.8 nmoles); ▒, ■−■ levorphanol (5.3 nmoles); ▩, ○−○ dextrorphan (5.3 nmoles), and ▦, □−□ codeine (5.3 nmoles). Vertical lines represent S.E.M. Significant differences ($p<0.05$) from dextrorphan are indicated as * and from codeine as ▼.

RESULTS

Experiment 1

Injection of levorphanol (1.8 and 5.3 nmoles) was followed by a short period of exploratory activity and then dose-dependent feeding which increased slowly during the 3-hour period to reach maximum levels of 1.2±0.6 g and 1.9±0.4 g, respectively (see Fig. 1). Cumulative food intakes both 2 and 3 hours after injection of 5.3 nmoles levorphanol, were significantly greater than those occurring after treatment with an equimolar quantity of dextrorphan: 2 hr—$F(3,15)=3.28$, $p<0.05$; 3 hr—$F(3.15)=4.36$, $p<0.05$. Little ingestion was apparent after codeine and the animals, although awake, remained fairly inactive; the weight of food consumed in the 3 hours following injection of codeine (5.3 nmoles) was significantly less ($p<0.05$) than following 5.3 nmoles levorphanol but not different from dextrorphan. There were no significant differences in core temperature among the four treatment groups.

Experiment 2

Only minimal ingestion followed injection of saline and ketocyclazocine (see Fig. 2). Feeding after the latter was occasionally preceded by a short (less than 10 min) period of depressed activity followed by sporadic nibbling and sleeping. There was virtually no food intake following phencyclidine. (Only 1 animal ate 0.1 g food during the third hour of the study). These animals assumed a natural sleep position immediately following injection but were arousable. Morphine evoked its previously noted effects [24]; there was an initial lengthy period of behavioural depression which lasted at least 30 minutes, followed by a prolonged period of food intake. Morphine stimulated food intake to a level (2.8±0.7 g) that was significantly greater: 2 hr—$F(3,18)=9.48$, $p<0.01$; 3 hr—$F(3,18)=11.66$, $p<0.01$, than that observed following injection of the other 3 agents. Moreover, only morphine elevated core temperature over the other substances: 1 hr—$F(3,18)=44.41$, $p<0.01$; 2 hr—$F(3,18)=47.54$, $p<0.01$; 3 hr—$F(3,18)=13.47$, $p<0.01$. The hyperthermia produced was observed for the duration of the study.

DISCUSSION

Levorphanol, which is a potent narcotic analgesic, stimulated feeding in a dose-related manner when injected into the ventromedial hypothalamus (VMH) of rats. On the guinea-pig ileum levorphanol is about 7 times more potent than morphine, a ratio which is similar to the analgesic potency in

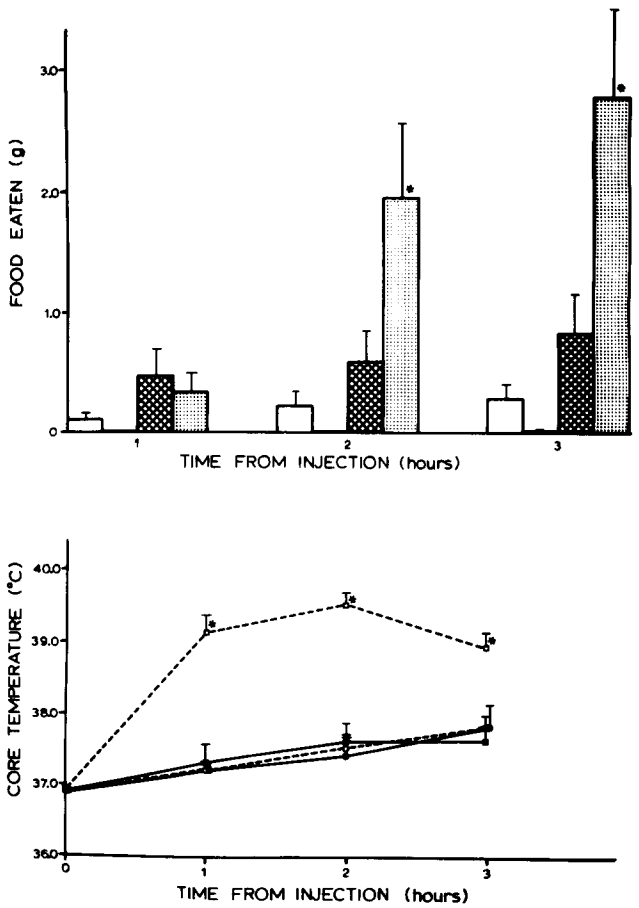

FIG. 2. Cumulative food intake and core temperature of rats (n=7) over 3 hours following administration into the ventromedial hypothalamus of □, ○−○ saline or 5.3 nmoles of: ○−○, ●−● phencyclidine; ▨, ■−■ ketocyclazocine; and ▩, □−□ morphine. Vertical lines represent S.E.M. Significant differences ($p<0.01$) from saline, phencyclidine and ketocyclazocine are indicated as *.

man [12]. In the present study, however, feeding elicited by levorphanol (Fig. 1) resembles that elicited by an equimolar dose of morphine (Fig. 2). As levorphanol is more lipophilic than morphine, it is possible that the difference reflects a faster diffusion of levorphanol from the receptor site into the surrounding tissue and hence an apparent reduction of potency relative to morphine. On the other hand, this equality of potency might reflect a difference in receptor type from that involved in analgesia. Nonetheless, since levorphanol induced significantly more feeding than its stereoisomer dextrorphan, narcotic analgesics appear to enhance food ingestion by acting on a stereoselective opiate receptor. The lack of effect of codeine on feeding behaviour further supports this, for it is a weak ligand for opiate receptors. It is known to alter feeding and thermoregulatory patterns in rats after peripheral injection but it is probable that these effects arise after hepatic biotransformation to morphine [10, 11, 27].

Only morphine and levorphanol caused a gradual increase in food ingested over the 3-hour experimental period. As implied above, it is probable that this response occurs after μ-opiate receptor activation. Phencyclidine, which has σ-opioid binding properties [9] and produces effects indistinguishable from the σ-opioid agonist SKF 10,047 in discriminative tests in rats [8], was without effect in this study. Ketocyclazocine, the prototype κ-opioid agonist analgesic [17], did not increase significantly the amount of food eaten when given directly into the VMH. This latter result contrasts with observations of others who have given κ-opiate ligands by a peripheral route; ketocyclazocine and ethylketocyclazocine given subcutaneously to rats both stimulated food intake as effectively as morphine [21,30]. This suggests that feeding behaviour after narcotic analgesics may be a complex phenomenon involving opiate receptors in other brain or peripheral sites.

We have observed consistently significant levels of hyperthermia in rats given central or peripheral injections of low doses of narcotic analgesics, and this hyperthermia occurred prior to, or concurrently with, the phase of stimulated feeding activity [24, 25, 27, 28]. Levorphanol, however, when given directly into the VMH, induced food intake without producing a parallel change in temperature. These observations imply that these two effects may not be integrally related.

From these studies, we propose that the opioid receptors in the VMH which can affect feeding are stereoselective and are responsive to μ-, but not κ- or σ-, agonists, Moreover, the feeding and hyperthermic responses in the VMH are not coupled effects. However, since both these parameters are responsive to μ-agonists such as morphine, yet are affected differentially by levorphanol, perhaps the μ-receptor has various subsets.

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