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The role of opioid receptor sub-types in tifluadom-induced feeding

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There is now considerable evidence that opioid agonists and benzodiazepines increase food and water intake in a variety of animal species. The appetitive effects of the novel opioid-benzodiazepine tifluadom have been investigated. (±)-Tifluadom significantly increased food intake in freely-feeding rats. This stimulation of appetite was attributable principally to the activity of the (+)-isomer. Furthermore tifluadom-induced feeding was blocked by the opioid antagonists naloxone, naltrexone, Mr 1452 and Mr 2266 but not by the δ-opioid receptor antagonist ICI 154, 129, or by the benzodiazepine antagonist Ro 15-1788. These results suggest that tifluadom exerts its effect on food intake by interaction with opioid as opposed to benzodiazepine receptors and that this activity is mediated by κ and/or μ- rather than δ-opioid receptor sub-types.

There has been growing evidence from animal behavioural studies for an involvement of endogenous opioids in the control of appetite (for reviews see Sanger 1981; Morley et al 1983a). In this context opioid agonists have been found to increase, whilst opioid antagonists decrease, eating and drinking in freely-feeding experimental animals. In addition, central administration of endogenous opioid peptides or their analogues have been shown to initiate feeding in satiated rats (Grandison & Guidotti 1977; McLean & Hoebel 1980; Morley & Levine 1981; Tepperman et al 1981).

In other studies a wide range of benzodiazepines has been shown to stimulate appetite and water consumption in a variety of animals (Cooper 1980). The novel compound tifluadom has recently been reported to increase food intake in non-deprived rats (Morley et al 1983b). This agent possesses a 1,4-benzodiazepine nucleus but has been shown to be active at opioid receptors in several in-vitro and in-vivo tests (Römer et al 1982a, b).

In the present study the mechanism of action of tifluadom-induced feeding has been investigated: firstly, by comparing the appetitive effects of (±)-tifluadom with those of its (+)- and (−)-isomers; secondly, by examining the effects of the opioid antagonists naloxone, naltrexone, Mr 1452, Mr 2266 and the benzodiazepine antagonist Ro 15-1788 on the appetitive activity of (±)-tifluadom. Finally, the possible involvement of enkephalinergic mechanisms in the feeding response to tifluadom was studied using the selective δ-opioid receptor antagonist ICI 154,129 (Shaw et al 1982).

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MATERIALS AND METHODS

Animals and environment

Male Wistar rats bred in the Welsh School of Pharmacy, 350–400 g at the time of experiment, were randomly allocated to groups of five or six and individually housed at 21 ± 1 °C in the animal house on a 12 h light/dark cycle. Before and during experimentation, animals were allowed free access to powdered standard rat diet (Grain Harvesters, Wingham, Kent) and tap water.

Food intake measurements

All experiments were commenced at 10.00 h so that measurements were carried out during the light period (08.00–20.00 h) when the food intake of control rats was minimal. Feeding jars were weighed at the time of drug administration and after 1, 2 and 4 h.

Drugs and injections

The following drugs were used: (±)-tifluadom (1-methyl-2(3-thienyl carbonyl)-aminomethyl-5-(2-fluorophenyl)-H-2,3-dihydro-1,4-benzodiazepine) and its (+)- and (−)-isomers, courtesy of Dr A. A. Badawy, Addiction Unit, Cardiff, and Dr D. Römer, Sandoz; naloxone and naltrexone, gifts from Endo laboratories; Mr 1452 ((−)-5,9-α-dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan) and Mr 2266 ((−)-5,9-α-diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan), courtesy of Dr. H. Merz, Boehringer Ingelheim; ICI 154,129 (*NN*-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH), gift from Dr R. Cotton, ICI Pharmaceuticals, and Ro 15-1788 (ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-

4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate), courtesy of Dr W. Haefely, Roche. All forms of tifluadom were dissolved in 10% ethanol; naloxone, naltrexone, Mr 1452 and ICI 154,129 were dissolved in sterile 0·9% NaCl and Mr 2266 was dissolved in 0·1 M HCl neutralized with $NaHCO_3$. Ro 15-1788 was suspended in distilled water to which Tween 80 was added (2 drops/10 ml). All drugs, except for ICI 154,129, were injected intraperitoneally in a dose volume of 1 ml kg^{-1}. For the ICI 154,129 injections rats were implanted with intracerebroventricular (icv) cannulae (2·5 mm lateral, 0·9 mm caudal to bregma; guide length 5 mm), and allowed at least 7 days to recover from the surgery before use. Injections were made directly into the lateral ventricle in a dose volume of 10 µl/rat.

In interaction studies animals were given 60 min pretreatments with Mr 1452 or Mr 2266 whereas all other antagonists were administered 30 min after (±)-tifluadom. In all cases treatment with corresponding vehicles did not significantly affect food intake.

Statistical analysis

The data are expressed as mean group cumulative food intake (g kg^{-1} rat weight). Standard errors of the group means were calculated and the difference between means was assessed by the Mann-Whitney 'U' test (two-tailed). Significance was assumed when P values of 0·05 or less were obtained.

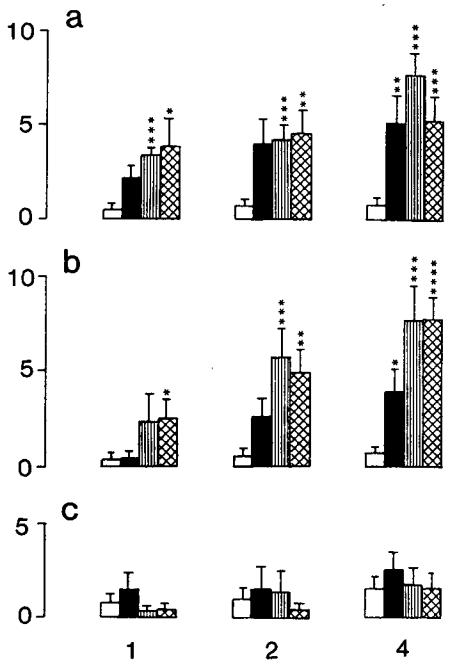

FIG. 1. Effect of (±)-tifluadom (a), (+)-tifluadom (b) and (−)-tifluadom (c) on food intake. Ordinates: cumulative food intake (g kg^{-1} rat weight). Abscissa: time after drug administration (h). Rats were injected i.p. with vehicle (open columns); 0·5 mg kg^{-1} tifluadom (closed columns); 1 mg kg^{-1} tifluadom (vertical-hatched columns) or 2 mg kg^{-1} tifluadom (cross-hatched columns). Significant differences from control values are indicated by $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}02$, $^{***}P < 0{\cdot}01$, $^{****}P < 0{\cdot}005$.

RESULTS

Effect of (±)-tifluadom and its optical isomers on food intake

The effects of (±)-tifluadom (0·5, 1 and 2 mg kg^{-1}) on food intake over 4 h are shown in Fig. 1a. All three doses produced a significant increase in food intake when compared with control values although this did not assume significance until 4 h after injection of the lowest dose. Animals did not display signs of overt sedation at any of the doses of tifluadom tested.

The effects on food intake of identical doses of the (+)- and (−)-optical isomers of tifluadom over 4 h are shown in Fig. 1b and 1c respectively. (+)-Tifluadom affected feeding in a manner closely resembling that of the racemate whereas the (−)-isomer appeared to be inactive in stimulating intake of food at all the doses tested.

Effects of various opioid antagonists on tifluadom-induced feeding

Naloxone (1 mg kg^{-1}) antagonized the marked increase in food intake induced by tifluadom (2 mg kg^{-1}) to levels that were not significantly different from controls over the period of testing (Fig. 2a). Similar results were obtained for naltrexone (1 mg kg^{-1}; Fig. 2b); Mr 1452 (5 mg kg^{-1}; Fig. 3a) and Mr 2266 (2 mg kg^{-1}; Fig. 3b). All these antagonists did not significantly affect food intake when administered alone.

In addition, ICI 154,129 (10 µg/animal icv) did not significantly antagonize ($P > 0{\cdot}05$) the increase in food intake produced by 2 mg kg^{-1} tifluadom over the duration of testing. Furthermore this dose of ICI 154,129 was devoid of any intrinsic anorectic activity (data not shown).

Effect of the benzodiazepine antagonist Ro 15-1788 on tifluadom-induced feeding

The significant increase in food intake produced by tifluadom was not blocked by a 10 mg kg^{-1} dose of the benzodiazepine antagonist Ro 15-1788 over the 4 h test. Treatment with Ro 15-1788 (10 mg kg^{-1}) alone did not significantly affect food intake (data not shown).

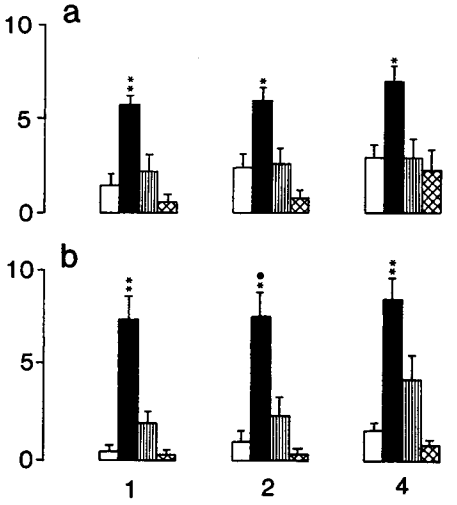

FIG. 2. Effect of naloxone (a; 1 mg kg^{-1}) and naltrexone (b; 1 mg kg^{-1}) on the food-intake induced by (±)-tifluadom (2mg kg^{-1}). Ordinates: cumulative food intake (g kg^{-1} rat weight). Abscissa: time after drug administration (h). Rats were injected i.p. with vehicle (open columns); tifluadom (closed columns); tifluadom and antagonist (vertical-hatched columns) or with the antagonist alone (cross-hatched columns). Significant differences from control values are indicated by $*P < 0{\cdot}01$, $**P < 0{\cdot}005$.

DISCUSSION

Several workers have recently reported that the novel benzodiazepine derivative tifluadom possesses activity at κ-opioid receptors (Römer et al 1982a, b; Upton et al 1983). Since these receptors have also been implicated in the initiation of feeding behaviours (Sanger & McCarthy 1981; Ferguson-Segall et al 1982; Morley et al 1982) this may account for the observation of Morley et al (1983b) that tifluadom increases food intake in non-deprived rats.

In the present study a similar increase in feeding was observed for (±)-tifluadom. Furthermore it appears that the (+)-isomer is mainly responsible for this effect. In radioligand binding studies using brain homogenates, (−)-tifluadom has been found to have a much greater affinity for opioid binding sites than the (+)-isomer (Kley et al 1983). These biochemical studies, however, refer to the optical rotation of the isomers dissolved in toluol which is the reverse of that displayed in ethanolic solution (Kley et al 1983). Consequently the (+)-isomer found to be active in our experiments when dissolved in ethanolic vehicle corresponds to the opioid-selective configuration described in toluol.

The feeding behaviour induced by tifluadom was effectively abolished by naloxone. Similar doses of antagonist reversed the food intake produced by morphine (μ) but not that produced by the κ-agonist ethylketocyclazocine (Sanger 1983). Moreover, Morley et al (1983b) found that a lower dose of naloxone inhibited the feeding response evoked by tifluadom though this antagonism was overcome at higher doses of the agonist. In our study, not only naltrexone, a longer acting antagonist than naloxone (Verebey & Mulé 1975), but also the benzomorphan structured antagonists Mr 1452 and Mr 2266, inhibited tifluadom hyperphagia. In-vivo Mr 2266 has been shown to be less potent in antagonizing morphine than naloxone but more potent than naloxone in antagonizing κ-agonists and it has been described by several workers as a selective κ-antagonist (Römer et al 1980; Shearman & Herz 1981; Ferguson-Segall et al 1982; Oka et al 1982; Leander & Hynes 1983). In some in-vitro studies however, Mr 2266 has been found to have equal activity at both κ- and μ-receptors (Lord et al 1977; Kosterlitz et al 1981) and thus the specificity of this agent is still a matter of controversy. Therefore it remains a possibility that the tifluadom-induced feeding response is due to activity at both μ- and κ-receptors.

ICI 154,129, found to act as a selective antagonist at δ-opioid receptors in the rat feeding model (Jackson & Sewell 1984), did not block the appetitive effect of tifluadom. It seems unlikely therefore that tifluadom-induced feeding involves either direct activity at δ-receptors or an indirect mechanism involving enkephalin release arising from an initial benzodiazepine action (Cooper 1983). This is further

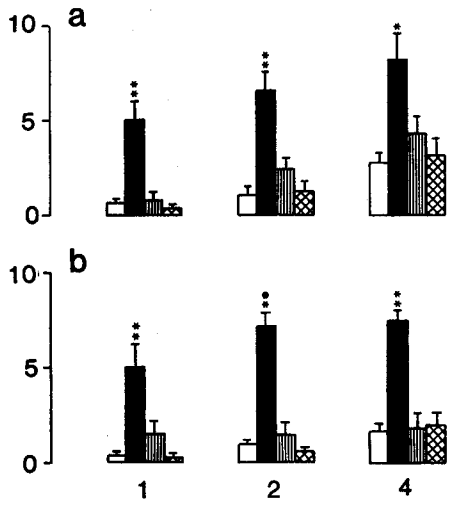

FIG. 3. Effect of Mr 1452 (a; 5 mg kg^{-1}) and Mr 2266 (b; 2 mg kg^{-1}) on the food intake induced by (±)-tifluadom (2 mg kg^{-1}). Ordinates, abscissa and drug treatment groups as in Fig. 2. Significant differences from control values are indicated by $*P < 0{\cdot}05$, $**P < 0{\cdot}005$.

substantiated by the finding that an effective dose of the benzodiazepine antagonist Ro 15-1788 (Hunkeler et al 1981) does not inhibit the stimulation of food intake by tifluadom and accords with reports that tifluadom has little affinity for benzodiazepine binding sites (Römer et al 1982a, b). However, it has been shown that (+)-tifluadom exhibits some affinity for the benzodiazepine receptor (Kley et al 1983). The corresponding isomer in ethanol had no effects on food intake in this study and this also indicates a lack of benzodiazepine involvement in tifluadom-induced feeding.

In conclusion the present findings suggest that the appetitive effects of tifluadom may involve activity at opioid receptors of the κ- and/or μ- but not δ-subtypes. The isomer responsible for this effect has previously been linked with opioid activity and in addition it would appear that benzodiazepine receptor interactions do not play any significant role in the initiation of feeding behaviour by this drug.

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