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An analysis of the effects of systemically administered clonidine on the food and water intake of rats

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1 It is known that intracerebral injections of clonidine can induce eating in rats but it has not been clear whether systemic administration can produce similar effects.

2 Subcutaneous injections of clonidine (0.01, 0.03, 0.1 mg/kg) increased food and water intake during the 6 h period following injection in non-deprived male rats.

3 Pretreatment with a dose of yohimbine (1.0 mg/kg) shifted the clonidine dose-response curves to the right, suggesting competitive antagonism.

4 A dose of naloxone (0.1 mg/kg) produced a lowering of the clonidine dose-response curve but statistical analysis suggested that the opiate antagonist did not produce a competitive antagonism of the effect of clonidine.

5 The results are consistent with a role for α_2-adrenoceptors in appetite regulation.

Introduction

Clonidine is an α-adrenoceptor agonist which is used clinically for its antihypertensive properties. In addition to its effects on cardiovascular function, clonidine exerts a number of behavioural actions (Laverty & Taylor, 1969; Fielding & Lal, 1981). After injection into the ventricles or hypothalamus, clonidine has been found to increase food intake in rats (Broekkamp & van Rossum, 1972; Ritter, Wise & Stein, 1975). Such findings are consistent with the view that central noradrenergic mechanisms have important functions in the control of appetite (Blundell & Latham, 1979; Leibowitz, 1980). However, there is some controversy as to whether systemically administered clonidine can also enhance either food or water intake. In some studies subcutaneous or oral administration of clonidine has been reported to produce only decreases in eating or drinking (Le Douarec, Schmitt & Lucet, 1971; 1972; Atkinson, Kirchertz & Peters-Haefeli, 1978). Mauron, Wurtman & Wurtman (1980), however, reported that intraperitoneal injections of clonidine at doses of 0.025–0.5 mg/kg caused rats to increase both total food intake and protein intake when they were allowed to select between diets with high or low protein concentrations. Also, Schlemmer, Casper, Narasimhachari & Davis (1979) found that daily intramuscular injections of a dose of clonidine increased food intake and body weight of stumptail macaques. This action of clonidine was blocked by the α_2-antagonist yohimbine but not by the α_1-antagonist prazosin, suggesting that the effect of clonidine was due to activity at α_2-adrenoceptors (Schlemmer, Elder, Casper & Davis, 1981).

The purpose of the present study was to analyse in greater detail the actions of clonidine on food and water intake in non-deprived rats. The drug was given by subcutaneous injection and food and water intake measured at several time periods. Experiments are also described in which interactions between clonidine and yohimbine or the opiate antagonist naloxone were studied. Naloxone was used because there is now substantial evidence to suggest that endogenous opioids are involved in mechanisms controlling eating and drinking (Sanger, 1981). It has also been suggested that appetite regulation may involve interactions between opioid and noradrenergic systems (Morley, 1980; Tepperman, Hirst & Gowdey, 1981; Morley, Levine, Murray & Kneip, 1982).

Methods

Animals

Male Sprague-Dawley rats (weighing 200–250 g) were used. They were housed individually and had

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free access to food (pelleted rat diet, Labsure) and to bottles containing tap water at all times. Each animal was used on only a single occasion.

General procedure

Each rat was removed from its home cage and given either one or two subcutaneous injections. After injection the animals were returned to their cages which contained weighed quantities of food and water and food and water intakes were assessed at different times after injection. Spilled food was collected in trays placed beneath the grid floors of the cages. Injections were administered at approximately 09 h 00 min and a non-reversed light cycle was in operation with lights on between 06 h 00 min and 18 h 00 min. At least nine rats were used in each condition.

In the first experiment, which studied the effects of clonidine when given alone, rats were injected with the drug at doses of 0.01, 0.03 or 0.1 mg/kg. Food and water intakes were then measured at 1, 2, 4, 6 and 24 h after injection. As it was found that food intake was maximally increased in the first 2 h and water intake in 4 h, the 2 h and 4 h measurement periods were used in subsequent experiments.

The purpose of the second experiment was to investigate whether the effects of clonidine were due to an action at α_2-adrenoceptors. A wider range of doses of clonidine was used than in the first experiment (0.003–0.3 mg/kg) and each dose was given immediately after an injection of saline or yohimbine (1.0 mg/kg). This dose of yohimbine was chosen on the basis of preliminary studies and as a dose which has previously been found effective in antagonizing other actions of clonidine (e.g. McCleary & Leander, 1981).

To study possible interactions between clonidine and the opiate antagonist, naloxone, a third experiment was carried out. Rats were injected with either saline or clonidine (0.003, 0.01 or 0.03 mg/kg) immediately after saline or naloxone (0.1 mg/kg). The naloxone dose was chosen as being at the threshold of doses which have previously been shown to reduce food and water intake induced by other procedures (Sanger, 1981). Also included in this experiment were a group of rats which had been injected with morphine at 1.0 mg/kg and a group which received both morphine and naloxone.

Drugs and doses used were clonidine HCl (0.003–0.3 mg/kg), yohimbine HCl (1.0 mg/kg) naloxone HCl (0.1 mg/kg) and morphine sulphate (1.0 mg/kg). All doses are expressed as base and injection volumes were 1 ml/kg body weight.

Statistical analysis of the data used 2 way analyses of variance, Dunnett's test and Student's *t* test.

Results

Effects of clonidine

After injection of clonidine in doses of 0.01, 0.03 and 0.1 mg/kg food and water intake were increased during the following 6 h period. Figure 1 presents cumulative food and water intake after saline or clonidine. Control animals showed very low levels of intake as would be expected of satiated rats tested during the morning. Food intake was increased by clonidine at all time periods and water intake was increased by 0.03 and 0.1 mg/kg at 4 and 6 h and there was also a small but statistically significant increase in water intake after 0.01 mg/kg at 2 h. At 24 h after injection the food intake of drug-treated rats was not different from that of the controls but in the animals which had received 0.1 mg/kg of clonidine the 24 h water intake was significantly above the control level (Table 1).

Interaction of clonidine and yohimbine

Figure 2 shows the effects of clonidine, given after either saline or yohimbine, on food intake in 2 h. In confirmation of the results of the first experiment, low doses of clonidine (0.003, 0.01 and 0.03 mg/kg) increased food intake while the highest dose given alone (0.1 mg/kg) produced no effect on this measure. Yohimbine alone also did not produce a statistically significant change in the amount of food consumed. However, Figure 2 indicates that yohimbine antagonized the action of clonidine as it appears to have shifted the whole biphasic dose-response curve to the right, suggesting competitive antagonism. These data were analysed using a 2-way

Table 1 The effect of clonidine on food and water intake in 24 h following injection

	Dose of clonidine (mg/kg s.c.)			
	Saline	*0.01*	*0.03*	*0.1*
Food intake (g)	28.2 ± 0.5	26.2 ± 0.8	27.3 ± 1.2	26.1 ± 1.1
Water intake (ml)	31.6 ± 0.7	32.2 ± 2.0	37.5 ± 1.9	39.1 ± 1.8*

Each value is the mean ± s.e. mean of food or water intakes of a group of 9 or 10 rats.
*$P < 0.05$ compared with saline.

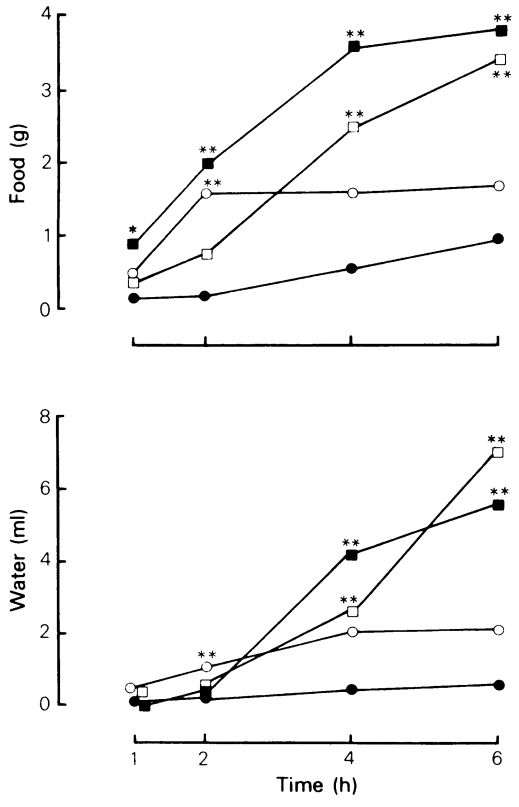

Figure 1 Cumulative food and water intake after subcutaneous injection of saline (●) or clonidine: 0.01 mg/kg (○); 0.03 mg/kg (■); 0.1 mg/kg (□). Each point is the mean cumulative food or water intake of a group of nine or ten rats. The animals had not been deprived of either food or water and the test began at 09 h 00 min. Statistical significance of differences are $*P<0.05$ and $**P<0.01$ compared with saline control.

(clonidine × yohimbine) analysis of variance. This showed a statistically significant effect of clonidine dose ($P<0.01$), no significant overall effect of yohimbine but a highly significant interaction ($P<0.001$). This can be taken as an indication that yohimbine produced a statistically significant shift to the right of the clonidine dose-response curve.

The effects of combinations of clonidine with the dose of yohimbine on water intake in the 4 h period after injection are illustrated in Figure 3. This figure shows that doses of 0.01 and 0.03 mg/kg of clonidine increased water intake and that the whole dose-response curve was shifted to the right by yohimbine. However, these data are more difficult to interpret unequivocally than the food intake data, because the dose of yohimbine when given alone increased water intake. An analysis of variance showed a statistically significant effect of clonidine ($P<0.01$) and a statistically significant interaction ($P<0.01$). A t test showed that yohimbine alone significantly increased intake ($P<0.05$).

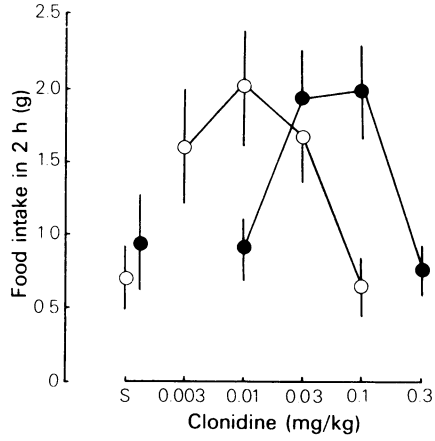

Figure 2 Dose-response curves showing the effects of clonidine injected immediately after saline (○) or yohimbine (●, 1 mg/kg) on the food intake of non-deprived rats in a 2 h period. Each point shows the mean food intake; vertical lines indicate s.e.mean. Yohimbine shifted the curve to the right.

Interaction of clonidine and naloxone

The effects of clonidine, morphine and naloxone on food intake in the 2 h period after injection are shown in Figure 4. It can be seen that the dose of morphine increased food intake ($P<0.05$) and that this action

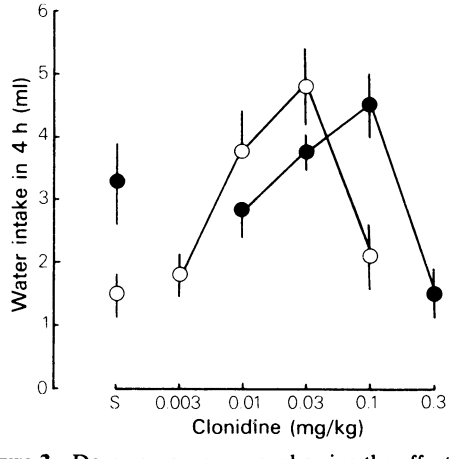

Figure 3 Dose-response curves showing the effects of clonidine injected immediately after saline (○) or yohimbine (●, 1mg/kg) on the water intake of non-deprived rats in a 4 h period. Each point shows the mean water intake; vertical lines indicate s.e.mean. Yohimbine shifted the curve to the right, but when given alone, itself increased water consumption.

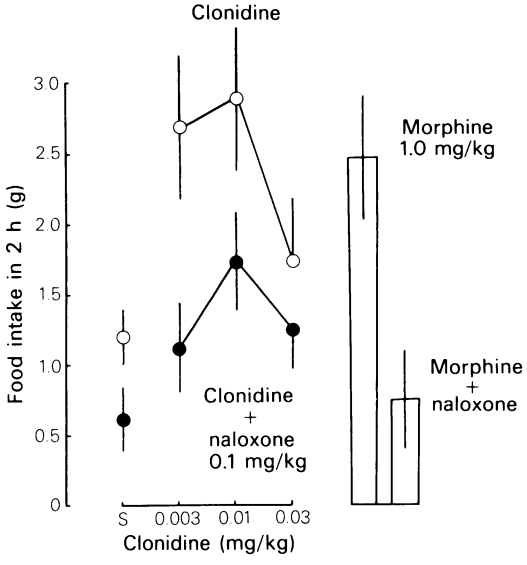

Figure 4 Dose-response curves showing the effects of clonidine injected immediately after saline or naloxone on the food intake of non-deprived rats in a 2 h period. Naloxone produced a general lowering of the clonidine dose-response curve, but did not prevent clonidine from increasing food intake. Also shown for comparison are the increase in food intake produced by a dose of morphine and the antagonism of this effect by naloxone. Each point or column shows the mean food intake; vertical lines indicate s.e.mean.

was antagonized by naloxone ($P<0.01$ difference between morphine alone and morphine and naloxone together). The data shown in Figure 4 also suggest that the food intake induced by clonidine was antagonized by naloxone. However, this antagonism appears different from that produced by yohimbine in that instead of naloxone causing the clonidine dose-response curve to be shifted to the right, it produced a general lowering of the curve although food intake in rats receiving both naloxone and clonidine was higher than intake in rats which received only naloxone. An analysis of variance showed a significant effect of clonidine dose ($P<0.001$), a significant effect of naloxone pretreatment ($P<0.01$) but no statistically significant interaction. These results, therefore, suggest that naloxone reduced food and water intake under all conditions but did not produce a specific antagonism of the food intake induced by clonidine.

The effects of clonidine and naloxone on water intake in the 4 h period after injection were similar to the results described above for food intake. The effect of clonidine was statistically significant ($P<0.001$) as was the effect of naloxone ($P<0.01$) but not the interaction. Because of the similarity of these results to those for food intake the water intake data have not been presented.

Discussion

The results of this study show that subcutaneous injections of low doses of the α-adrenoceptor agonist, clonidine, can produce increases in levels of food and water intake in rats. Food intake was increased in the first and second hour after injection and remained above control levels at 6 h. Increases in water intake appeared, in general, to occur at later time periods than increases in food intake and after 0.1 mg/kg water intake remained significantly above control values for 24 h. It has previously been suggested that this long lasting action on drinking may be due to the diuretic effect of clonidine (Atkinson *et al.*, 1978).

The increased food intake confirms the results of previous studies which have shown that intracerebral injections of clonidine can induce eating in rats (Broekkamp & van Rossum, 1972; Ritter *et al.*, 1975). It has also been shown previously that clonidine, given by intramuscular injection, can increase eating and body weight in monkeys but other studies have failed to observe increases in food or water intake after systemic injection in rats. Le Douarec *et al.* (1971, 1972) found that clonidine reduced food and water intake in food or water deprived rats. More recently, Atkinson *et al.* (1978) found no increases in food intake at 6 and 24 h after injection in freely feeding rats. It is not clear why the present results differ from those reported by Atkinson *et al.* (1978). One possibility may relate to the diets used. Mauron *et al.* (1980) have reported that clonidine can increase the intake of a high protein diet in rats allowed to select their diet from two food sources containing different concentrations of protein. Also, Leibowitz (1980) found that clonidine can produce a selective increase in carbohydrate intake. In both the present study and that described by Atkinson *et al.* (1978) standard rat diets were used. It is conceivable that these diets differed sufficiently to account for the different effects of clonidine.

Administration of a dose of yohimbine produced an antagonism of the clonidine-induced increases in food and water intake. Yohimbine caused the dose-response curves produced by clonidine to be shifted to the right suggesting a competitive pharmacological antagonism. As yohimbine is believed to act at α_2-receptors, this result suggests that the increases in eating and drinking produced by clonidine may be due to action at such receptors. This interpretation is complicated, in the case of water intake, by the finding that yohimbine itself increased drinking. Schlemmer *et al.* (1981) have also reported that the increase in food intake induced by a dose of clonidine

in monkeys is antagonized by a dose of yohimbine. The present study extends these previous results to a second species and by using a wide range of clonidine doses. It has also been shown previously that other behavioural effects of clonidine, including reductions in locomotor activity and rates of operant responding and increased latencies to react to nociceptive stimuli can be antagonized by yohimbine (Drew, Gower & Marriott, 1979; McCleary & Leander, 1981).

In the present study, experiments were not carried out to investigate interactions between clonidine and drugs such as prazosin, corynanthine or phenoxybenzamine believed to act preferentially at α_1-receptors. It is therefore possible that α_1-receptors may also have been involved in the observed effects of clonidine. However, most previous studies have found that other behavioural effects of clonidine, while being blocked by α_2-antagonists such as yohimbine, are not antagonized by α_1-antagonists (LeDouarec *et al.*, 1971; 1972; Hunt, Atrens, Baker & Paxinos, 1978; Drew *et al.* 1979; Reinstein & Isaacson, 1981). Also, it has been reported that the increased food intake induced in monkeys with a dose of clonidine is not antagonized with a dose of prazosin (Schlemmer *et al.*, 1981).

The effects of clonidine on food and water intake were similar, both qualitatively and quantitatively, to the enhanced eating and drinking produced by morphine and other opiate agonists under similar experimental conditions (Sanger & McCarthy, 1980; 1981). Recent research has suggested that there may be interactions between opioid and α-adrenergic mechanisms in the central nervous system and clonidine has been used clinically to treat withdrawal from opiates (Gold & Pottash, 1981). It has also been proposed that adrenergic and opioid systems may interact in the central control of appetite (Morley, 1980). Consistent with this proposal, Tepperman *et al.* (1981) have reported that morphine-induced eating can be reversed by phentolamine and Morley *et al.* (1982) have recently presented results indicating that relatively high doses of naloxone can block the eating induced by an intracerebral injection of noradrenaline.

In the present study an attempt was made to antagonize the effects of clonidine with a relatively low dose of naloxone (0.1 mg/kg) which, however, blocked the increase in food and water intake produced by a dose of morphine. Levels of food and water intake were higher in rats injected with only clonidine than they were in animals given both clonidine and naloxone. However, the statistical analysis indicated that, although both clonidine and naloxone each produced statistically significant effects, the interaction between the two drugs did not reach an acceptable level of statistical significance. Thus naloxone did not produce a competitive antagonism of the effects of clonidine similar to that produced by yohimbine. These results, however, are consistent with the involvement of both α-adrenergic and opioid systems in the control of food and water intake. It is also possible that, had higher doses of naloxone been used in the present study, a competitive antagonism between clonidine and naloxone might have been observed. This seems unlikely because such doses would be expected to produce greater reductions in control levels of intake and, in any event, high doses could act through non-opioid mechanisms. It has also recently been shown that naloxone blocks neither the effects of clonidine on punished and unpunished operant responding (Kruse, Dunn, Theurer, Novick & Shearman, 1981) nor its actions on locomotion and body temperature in infant rats (Reinstein & Isaacson, 1981). In unpublished results from the present author's laboratory, naloxone did not antagonize the reduced locomotor activity produced by clonidine in rats.

In summary, the present results showed that subcutaneous injections of clonidine can produce increases in food and water intake in non-deprived rats. These effects were antagonized by a dose of the α_2-adrenoceptor antagonist yohimbine, but not by a dose of naloxone. Thus these results are consistent with a role for noradrenergic, and particularly α_2-adrenoceptor, mechanisms in the control of appetite.

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