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Effects of Intracerebroventricular Injection of Naloxone on Operant Feeding and Drinking in Pigs

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BALDWIN, B A AND R F PARROTT *Effects of intracerebroventricular injection of naloxone on operant feeding and drinking in pigs* PHARMACOL BIOCHEM BEHAV 22(1) 37–40, 1985 —Operant feeding and drinking to satiation were studied in prepubertal pigs deprived of food or water for 18 hours and then given intracerebroventricular (ICV) injections of a solution of naloxone hydrochloride In feeding tests there was no difference in the amount of food consumed, or in the rate at which reinforcements were obtained, between pigs given ICV injections of 0 4 or 0 8 mg naloxone and those receiving a control injection of saline However, in drinking tests, injection of both 0.2 and 0 4 mg naloxone significantly ($p<0.01$) reduced the quantity of water drunk and slowed the rate at which reinforcements were obtained No significant effects on operant water intake were seen after intravenous injection of 0 4 mg naloxone

Pigs Naloxone ICV injection Operant feeding and drinking

THE isolation of opiate-like peptides from brain tissue [9] has stimulated a considerable research effort into the characterization and possible functions of endogenous opiate systems. An important factor in many of these investigations has been the use of specific opiate antagonists, such as naloxone [3]. This substance was first shown by Holtzman [8] to affect behavior by reducing food intake in deprived rats. Subsequently, numerous reports have shown that peripherally administered opiate antagonists will decrease both food and fluid intake in a variety of species and experimental situations (see [15,22] for reviews).

Opiate antagonists, like naloxone and naltrexone, may produce their behavioural effects by acting centrally, since forms of these agents which do not cross the blood-brain barrier are without effect [4,5]. However, there have only been a few studies, all carried out in rats, in which the central effects of opiate antagonists on behaviour have been investigated directly by means of intracerebroventricular (ICV) injection. It has been reported that ICV injection of naloxone, in doses ranging from 0.015 to 0 4 mg, reduced feeding in food-deprived animals [11, 26, 28]. Other studies have shown that ICV injection of 0.25 mg naloxone bilaterally [23] or 0 1 mg unilaterally [24] decreased drinking in water-deprived animals. However, in one investigation, where peripherally administered naloxone reduced both food and water intake, ICV injections in doses ranging from 0.0005 to 0.5 mg were without effect [10]

Investigations of the central actions of opiate antagonists in species other than the rat could be of value in the wider interpretation of the possible role of endogenous opiates in the control of ingestive behaviour. In this laboratory, the young pig has been used extensively in studies on the central and peripheral factors regulating food intake and methods have been developed for the introduction of substances into the lateral cerebral ventricles. We have, therefore, examined the effects of ICV injection of naloxone in this species and, as in previous studies [18,19], feeding and drinking were quantified by operant methods. We realize that the effects of pharmacological agents on operant and consummatory behaviours may differ, but in young pigs, operant methods prolong the duration of ingestive behaviour and facilitate its measurement

METHOD

Animals

The effect of ICV injection of a solution of naloxone hydrochloride ('Narcan,' Dupont (U.K.) Ltd., Stevenage, Herts.) on operant feeding and drinking was studied using 9 female and 2 male prepubertal Large White pigs. The animals lived separately in pens fitted with commercial drinkers and were floor fed daily with a standard ration of pelleted food. Before the experiments began, the pigs were trained to press a switch panel with their snouts, on a fixed ratio of 10, for reinforcement with either 12 g pelleted food or 8 ml water. Training and experimental testing took place in a pig trolley modified so that food or water could be automatically delivered into a bowl situated below the switch panel.

Surgery

The pigs were surgically prepared with guide tubes for ventricular cannulae, using a previously described method [18]. However, an improved connection system was employed to avoid the frequent replacement of catheters damaged by abrasion. Each cannula was screwed into a right-angled connector to which was attached a 12 cm length of

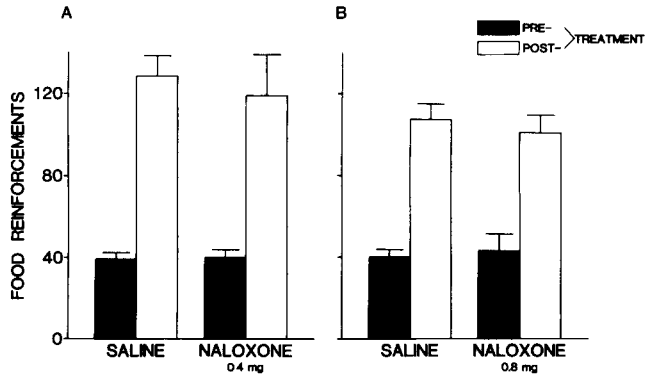

FIG 1 Operant food intake in 18 hour food-deprived pigs given ICV injections of, A, saline or 0 4 mg naloxone (Experiment 1) and, B, saline or 0 8 mg naloxone (Experiment 2) Number of food reinforcements (mean±S E) are shown for the 10 minute pre-treatment period and for the post-treatment period (i e , the interval from injection to satiation)

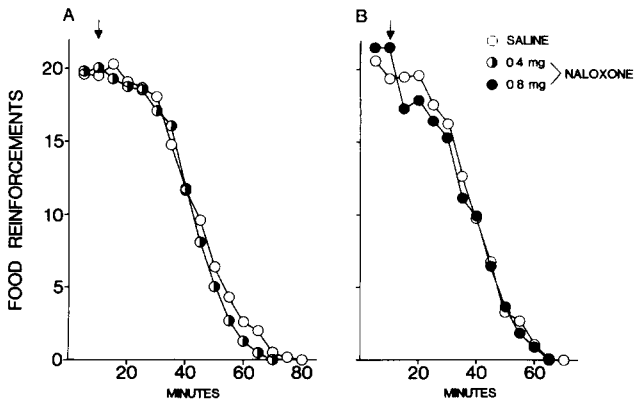

FIG 2 Operant food intake, expressed as the mean number of reinforcements obtained per 5 minute period, in 18 hour food-deprived pigs given ICV injection of, A, saline or 0 4 mg naloxone (Experiment 1) and, B, saline or 0 8 mg naloxone (Experiment 2) The arrow indicates the time when the injection was given

thick walled silastic tubing ending in a Luer fitting. This catheter tubing rested loosely on the animal's neck without any additional means of attachment and was readily available for the administration of injections. The locations and depths of the cannulae were established as described previously [18]

Procedure

Two feeding experiments were carried out with most of the animals participating in both studies In these experiments, the pigs were fed at 17.00 hr and testing commenced at 11.00 hr the next day, i.e., after a period of 18 hours food deprivation but with water available ad lib. Testing involved placing a pig in the trolley and allowing it to work for food When 10 minutes had elapsed, the ICV injection was given by hand and the pig was allowed to continue responding for food until it was satiated. In all experiments, a state of satiation was defined as having been reached when there was a 3 minute interval in which no reinforcements were obtained by the animal

In the first feeding experiment (Experiment 1), the pigs (N=6, 4 male, 2 female; mean weight 43 kg) were given ICV injections of either, 2 ml sterile normal saline (control) or, 1 ml 'Narcan' (0.4 mg naloxone hydrochloride) followed by 1 ml saline In the second experiment (Experiment 2), the pigs (N=5, 3 male, 2 female, mean weight 53 kg) received either, 3 ml saline (control) or, 2 ml 'Narcan' (0 8 mg naloxone hydrochloride) followed by 1 ml saline. The feeding behaviour of the animals was monitored on a cumulative chart recorder and the number of reinforcements obtained during each 5 minute period of the test was calculated for each animal

A drinking experiment (Experiment 3) was carried out using a different group of pigs (N=5 male, mean weight 62 kg) In this study, food was provided twice daily at 09.00 hr and at 16.00 hr and the animals were deprived of water from 17.00 hr to 11.00 hr, i e , for 18 hours. The method of testing was similar to that described above. The pigs were given ICV injections of 2 ml saline (control) or 0.5 ml 'Narcan' (0 2 mg naloxone hydrochloride) followed by 1.5 ml saline or, 1 0 ml 'Narcan' (0.4 mg naloxone hydrochloride) followed by 1 0 ml saline The injections were administered 4 minutes after the beginning of the test and the number of reinforcements obtained by individual pigs during each 2 minute period were calculated from the cumulative records After testing, the pigs were returned to their pens where they had access to water from their drinkers until 17 00 hr

In all the experiments the pigs were tested daily with the treatments given in rotating order In the majority of cases each pig received a given treatment three times during the course of an experiment The results obtained from these replications were averaged to provide treatment means for each pig and comparisons between treatments were made using the paired '*t*' test (2-tailed)

RESULTS

Food Intake

The two parts of Fig 1, (A and B), respectively show the total number of food reinforcements consumed by the pigs in the pre- and post-treatment periods of Experiments 1 and 2 In Experiment 1 (Fig. 1A), there was no difference in the number of reinforcements obtained in the 10 minute pre-injection period between the control and the naloxone-treated group. However, the groups also did not differ in the number of reinforcements consumed during the period from injection to satiation Similarly, in Experiment 2 (Fig 1B), there were no differences between treated and control groups in the number of reinforcements obtained in either the pre- or post-treatment periods

The mean number of reinforcements delivered in each 5 minute period of Experiments 1 and 2 shown in Fig 2, (A and B), respectively The rate at which the food was consumed was similar in both experiments and there were no obvious effects attributable to naloxone treatment

Water Intake

Figure 3 illustrates the total number of water reinforcements obtained in Experiment 3 in the 4 minute pre-injection interval and during the period from injection to satiation Pre-treatment totals were similar under the three experimental conditions but, in contrast to Experiments 1 and 2 (Fig 1, A and B), naloxone produced statistically significant ($p<0$ 01) dose-dependent reductions in the amount of water

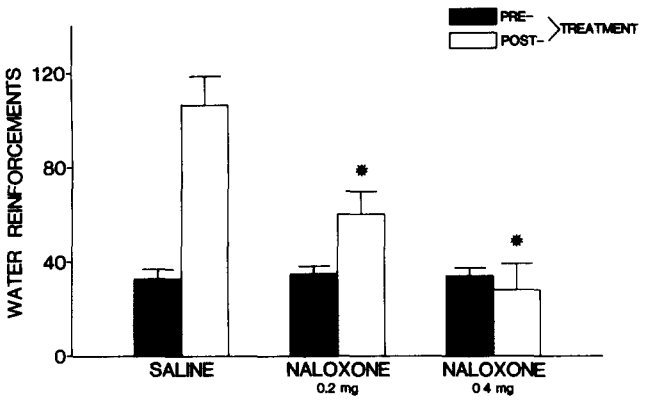

FIG 3 Operant water intake in 18 hour water-deprived pigs given ICV injections of saline or two doses of naloxone (Experiment 3) Number of water reinforcements (mean±S.E) are shown for the 4 minute pre-treatment period and for the post-treatment period (i.e , the interval from injection to satiation) (*$p<0.01$ vs saline control)

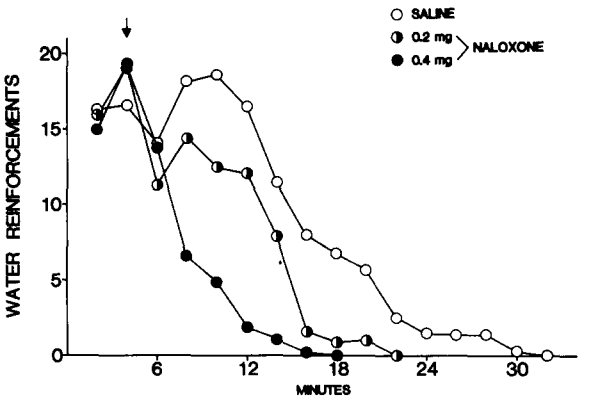

FIG 4 Operant water intake, expressed as the mean number of reinforcements obtained per 2 minute period, in 18 hour water-deprived pigs given ICV injections of saline or two doses of naloxone (Experiment 3) The arrow indicates the time when the injection was given

drunk during the post-treatment period. There was no sign of discomfort or illness following administration of naloxone.

The mean number of water reinforcements obtained in each 2 minute period of Experiment 3 are shown in Fig.4. It can be seen that, unlike the situation where the pigs were working for food (Experiments 1 and 2, Fig. 2), there was a tendency for the rate at which reinforcements were obtained to decrease in the period immediately following the injection. However, whereas in the control group this decrease was followed by an increased work rate, when the pigs were given 0.2 mg naloxone there was a much smaller recovery, and treatment with 0.4 mg naloxone was followed by a sharp decline. Furthermore, the dose-related action of naloxone on the total number of reinforcements obtained in the post-injection period (Fig.3) is reflected in Fig. 4 by a similar effect on the time taken to reach satiation. Inspection of the cumulative records provided an estimated duration of action of naloxone of between 2 and 4 minutes for the 0.2 mg dose and about 8 minutes for the 0 4 mg dose.

The effects of water intake seen in Experiment 3 may have been due to leakage of naloxone from the ventricles into the peripheral circulation. Therefore, four pigs (3 male, 1 female; mean weight 24 kg) were prepared with jugular catheters and the effects on water intake of intravenous injection of naloxone were examined. The dose used was 0.4 mg which, when given ICV, produced a marked reduction in water intake (Figs. 3 and 4) This dose was flushed in with 4 ml of normal saline The control procedure was to inject 5 ml of saline. The time of injection and the experimental procedures were as in Experiment 3 The results obtained are outlined below

The number of water reinforcements (mean±SE) delivered during the 4 minute pre-treatment period was similar under experimental and control conditions (23.7±4.2, saline; 23 7±3.5 naloxone). During the post-treatment period the pigs obtained 65.9±18 5 (mean±SE) reinforcements after saline injection compared with 54 7±13.1 (mean±SE) after naloxone. This difference was not significant.

DISCUSSION

In pigs deprived of food or water for an equivalent period, ICV naloxone injection produced a dose-related decrement in operant drinking but did not affect operant feeding, even when given at a higher dose. Although the ventricular volume of the pig has not been determined, the brain weight is similar to that of the goat where the ventricles are reported to contain 8–12 ml CSF [17] Taking into account the much larger volume of CSF in the pig, the effective dose of naloxone (0 2 mg) would appear, in terms of CSF concentration, to be lower than the doses reported to reduce consummatory drinking in the rat (0.05 mg, 23, or 0.1 mg, 24). The present study also shows that the higher of the two ICV doses that affected operant drinking (0 4 mg) failed to produce a similar effect after IV administration.

Several workers (reviewed in [15]) have found that animals deprived of water are more sensitive to the effects of peripherally administered naloxone than those deprived of food. Similarly, in the present study, although the periods of food and water deprivation were the same, ICV injection of naloxone affected drinking but not feeding However, this difference may be due to different levels of motivation in the two test situations and this consideration may also explain some of the variable effects on feeding and drinking reported in other species Certainly, the presentation of food after 18 hours deprivation elicits excitement and vocalization in young pigs whereas the same response is not seen when water is made available. The motivation to work for food is also much greater than for water and operant drinking, unlike operant feeding, is more easily disrupted by interference, as indicated by the effect of the control injection (Fig. 4). These effects are probably related to the hyperphagic nature of the young pig. This is supported by the finding that lesions in the ventromedial hypothalamus induce hyperphagia in mature pigs [1] but not in young animals (Auffray, personal communication)

In studies with rats where drinking was reduced by ICV naloxone injection [23,24], doses that were effective centrally were not effective peripherally. In the present experiments, intravenous injection of a dose (0 4 mg) of naloxone that markedly reduced operant drinking when given ICV (Experiment 3) tended to reduce drinking but the effect was not significant. However, this tendency should be considered in relation to body weight The pigs in Experiment 3 received an ICV dose of 6.5 μg/kg whereas the animals treated intravenously received 16 7 μg/kg. Taken together,

these results demonstrate that naloxone can exert a central effect which reduces drinking in young pigs However, the fact that the effective ICV dose (0 4 mg), when given intravenously tended to reduce water intake, may indicate that naloxone can act at both central and peripheral sites in pigs.

The behavioural effects of naloxone are diverse because, in addition to reducing deprivation-induced eating and drinking, it also decreases feeding induced by insulin [13, 16, 21] and 2-deoxy-D-glucose [16], drinking in response to hyperosmolarity, hypovolemia and angiotensin II [20], and self-stimulation [2] In addition, naltrexone, a related compound, has been shown to depress sexual activity in primates [14] Although it is difficult to conceive of a common physiological basis for such a plethora of effects, the explanation may be to do with modulation of brain mechanisms of reward [2] However, the following lines of evidence suggest an alternative interpretation

The assumption in the majority of studies is that naloxone produces its behavioural effects by interference with endogenous opiate (endorphinergic) systems modulating ingestive activity [15,22] However, it is conceivable that it may have effects unrelated to its action as an opiate antagonist and a recent report has shown that ICV naloxone at high doses can actually have morphine-like effects [6] Finally, in the light of several reports indicating that naloxone can produce taste aversions [12, 25, 27] and its clearly aversive action in the cat [7], the possibility exists that, in some species, it influences a variety of behaviours by producing some subtle form of malaise However, no signs of illness or discomfort were seen in the present study

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