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Effects of Naloxone and Its Quaternary Form on Fluid Consumption in Rats

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HEMMER, R. C., G. A. OLSON, A. J. KASTIN, J. H. McLEAN AND R. D. OLSON. *Effects of naloxone and its quaternary form on fluid consumption in rats.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1287–1290, 1982.—Three studies were performed on albino rats to determine the effects of naloxone and its quaternary derivative, naloxone methylbromide, on fluid consumption. The doses of the quaternary naloxone were equated with naloxone by molarity and effectiveness in order to facilitate direct comparisons. All rats were deprived of food and water for 12 hr and exposed to a 20% sucrose solution for a 2 hr period. In Experiment 1, a low (0.01 mg/kg) dose of naloxone or an equated dose of quaternary naloxone was given ICV and immediate access allowed to the fluid on four consecutive days. Animals receiving naloxone were not significantly different from controls, and rats receiving quaternary naloxone exhibited seizures, resulting in decreased consumption. In Experiment 2, the low dose of naloxone or the equated dose of quaternary naloxone was given IP for four consecutive days and neither was significantly different from controls. In Experiment 3, animals were given an IP dose of either 1 mg/kg naloxone, a 1 mg/kg or 50 mg/kg dose of quaternary naloxone, or saline and tested for a single 2 hr period. The doses of 1 mg/kg naloxone and 50 mg/kg quaternary naloxone produced significantly less drinking than controls. In all studies, the initial 30 min period produced the most drinking. Suppression of drinking by a dose of 50 mg/kg quaternary naloxone suggested, in contrast to other studies, that it may cross the blood-brain barrier at high doses.

Naloxone methylbromide Opiate antagonists Drinking

THE opiate antagonist naloxone (NAL) has become a valuable tool in elucidating the role of the endogenous opiates in many types of behaviors. The agonistic and antagonist actions of endogenous opiate and nonopiate compounds have been implicated in the modulation of consummatory behavior. It has been found that β-endorphin administered either intrahypothalamically [12] or intraventricularly [17] stimulates feeding in both food-deprived and satiated rats. Furthermore, high levels of β-endorphin in the pituitary and plasma of genetically obese strains of rats and mice have been associated with overeating in these strains, and low doses of NAL suppress this feeding activity [18]. Numerous studies have shown that NAL, when administered either systemically or centrally, decreases both food and fluid consumption [2–8, 12, 14–16, 21, 23]. The site of action of NAL is unclear, although evidence attributes its effects to a central mechanism [6, 10, 27, 28].

Recent research with a quaternary form of naloxone, naloxone methylbromide, (Q-NAL) has shown that this substance does not produce the characteristic decrease in fluid consumption that NAL does when injected systemically [6]. Thus, a central site of action has been proposed on the basis that the blood-brain barrier is impermeable to Q-NAL. Other studies with Q-NAL have shown it to be 20–65 times less potent than NAL in displacing stereospecific ^{3}H-etorphine binding in rat brain membranes and in antagonizing the effects of morphine on the isolated guinea pig ileum [28]. Also, in narcotic-naive and narcotic-dependent pigeons trained to discriminate the opiate antagonist naltrexone from saline, Q-NAL and Q-naltrexone both consistently failed to produce stimulus control of the behavior when injected peripherally [28]. Other evidence has shown that systemically administered Q-NAL is ineffective in altering jump escape latencies in stress-induced analgesia and that equimolar doses of Q-NAL failed to effect morphine-induced analgesia in rats after peripheral injections [11]. Central injections of Q-NAL have been shown to suppress drinking at a dose of 10 μg [6]. Thus it has been proposed that Q forms of opiate antagonists, while acting similarly, do not permeate the blood-brain barrier and can be used to differentiate between a central or peripheral site of action [6].

The purpose of this study was to compare the effects of equimolar doses of NAL, Q-NAL and saline control on fluid consumption in rats. A comparison of the effects of these compounds at a central (intraventricular, ICV) and peripheral (intraperitoneal, IP) site of injection at both high and low doses could provide evidence to help establish the site of action of opiate antagonists.

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EXPERIMENT 1

METHOD

The effects of ICV injection of a low dose (0.01 mg/kg) of NAL and Q-NAL were tested. Some evidence exists which shows that a low dose of naloxone increases fluid consumption overall [21], or at least in some instances [3,7].

Animals

Male rats (n=20) derived from the Sprague-Dawley strain were obtained from King Laboratories (Oregon, WI) and were housed individually in a temperature controlled colony (22–24°C) with a 12 hour light-dark cycle (light onset at 0800 hr) throughout the experiment. These naive rats weighed an average of 380.0 g at the start of the study. The animals were handled daily to familiarize them with the handling involved in the ICV injection and the maintenance of the cannula.

Drugs

The NAL (Endo Laboratories) and Q-NAL (Boehringer und Sohn) were calculated on an equimolar basis (0.01 mg/kg NAL and 0.0118 mg/kg Q-NAL). This dose of Q-NAL was then multiplied by 42.5 to arrive at the injected dose of 50 mg/kg since several studies have shown that polar quaternary derivatives are 20–65 times less potent and this factor is midway between the two extremes [11, 27, 28]. Both drugs were tested with their own isotonic saline controls to facilitate statistical comparisons. Each animal received one of four coded solutions: 0.01 mg/kg NAL, 0.0 mg/kg saline, 0.5 mg/kg Q-NAL or 0.0 mg/kg saline. Since all injections were given ICV in this experiment, the injection volume was only 1 μl/100 g of rat. The drug condition was studied in a mixed design and randomized order.

Surgery

The rats were allowed free access to food and water for 10 days before the cannulation procedure. After this habituation period, the rats were anesthetized with a 0.7–0.8 ml Ketaset (Bristol Laboratories) and the head was shaved. The rat was placed in a stereotoxic instrument (David Kopf Instruments) and a 2 cm incision was made on the midline from a point between the eyes caudal to the occipital pole. The skull was exposed and the bregma located and used as stereotoxic zero. The guide cannulae (No. 313-G Plastic Products Co., Roanoke, VA) were cut so that they would extend to a point one mm above the middle of the left cerebral ventricle and the "dummy" (No. 313-DC) and injection (No. 313-I) cannulae were cut to extend 1.0 mm past the tip of the guide cannulae. Four holes were drilled into the skull, three for screw placement and one for the guide cannulae which was positioned at coordinates of +2.0 mm on the medial-lateral arm and −0.7 mm on the anterior-posterior arm from the bregma. Guide cannulae were secured with cranioplastic cement (Plastic Products Co., Roanoke, VA). The incision was sutured and the "dummy" cannula placed in the guide cannula. The animal was allowed a 10 day recovery period during which the "dummy" cannula was cleaned daily in an alcohol bath. During this time, rats were allowed free access to food and water.

Procedure

At 2000 hr all water and food were removed from the cages and the rats were given 8 g of food. Starting at 0800 hr the animals were weighed and any remaining food was removed from the cages. The drugs were infused using a Gastight 1705 microliter syringe (Hamilton Co., Reno, NV) over a 30 sec period, with the average volume being 3.8 μl. This rather quick injection as compared to other studies [5] was used since a rapid onset of effects was expected. The rats did not exhibit any behavioral effects due to the ICV injection. After the injection, the animals were returned to their cages and allowed immediate access to a 20% sucrose solution. Recordings of fluid intake were made every 30 min for a 2 hr period. As soon as the 2 hr test period was completed, the animals were allowed free access to food and water until the deprivation period began at 2000 hr. The animals were tested in this manner for four consecutive days. Upon completion of testing, the animals were sacrificed and proper placement was confirmed in each case with standard histological procedures.

RESULTS

A mixed analysis of variance (drug × dose × day × interval) was performed on the amount of fluid consumed. The main effect for drug was significant, $F(1,16)=11.546$, $p<0.01$, and is attributable to the observation that 4 out of 5 subjects in the Q-NAL group exhibited seizures throughout the study with the fifth animal exhibiting seizures on the last (fourth) day. The seizures did not totally interfere with drinking activity, but its effects were powerful enough to alter most factors in this analysis. The main effect for dose was reliable, $F(1,16)=7.68$, $p<0.05$, $F(1,16)=6.764$, $p<0.05$, along with the interaction of these 2 factors (drug × dose). The main effect for time interval was highly significant, $F(3,48)=297.871$, $p<0.00001$, along with interactions of interval × drug, $F(3,48)=16.603$, $p<0.00001$, interval × dose, $F(3,48)=7.092$, $p<0.001$, and the three-way interaction, drug × dose × interval, $F(3,48)=6.225$, $p<0.01$. All significant factors seem to be related to the seizure activity which interfered with drinking as there was no difference between the NAL group and the two saline control groups. The three-way interaction showed that after the initial interval, drinking behavior was similar in all groups.

EXPERIMENT 2

METHOD

The effects of an intraperitoneal (IP) injection of a low dose of naloxone of Q-NAL were examined.

Animals

Male rats (n=20) were obtained from King Laboratories and maintained and handled exactly as described in Experiment 1. The average weight of the experimentally naive animals was 295.0 at the start of testing.

Drugs

Animals received either 0.01 mg/kg NAL, an equimolar (0.5 mg/kg) dose of Q-NAL with appropriate adjustments (see Experiment 1), or an equivalent amount of saline for the two control groups. The drug condition was randomized within subjects.

Procedure

The procedure was the same as for Experiment 1 except that an IP injection rather than ICV infusion was used.

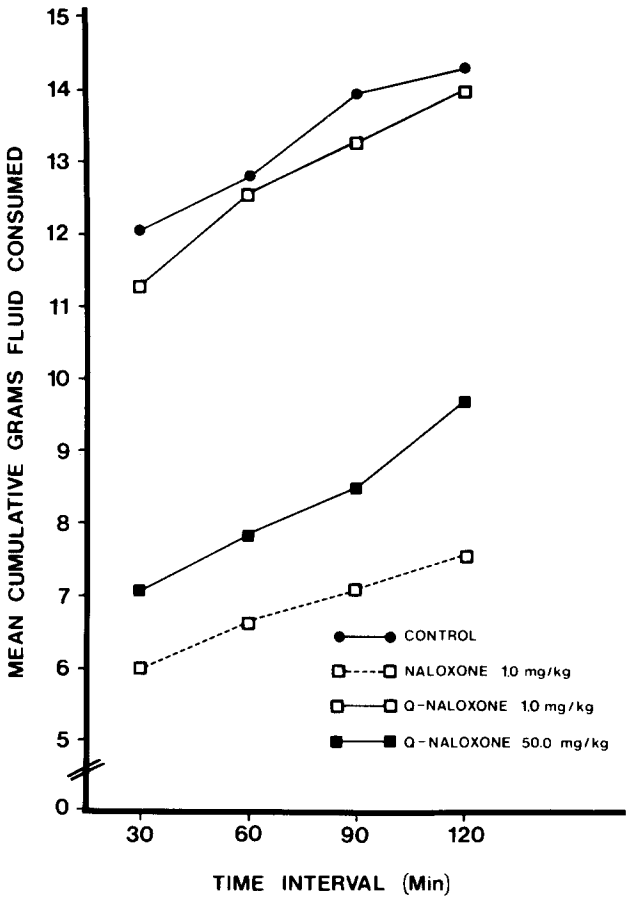

FIG. 1. Mean cumulative grams of fluid consumed in a single two hour test session.

RESULTS

A mixed analysis of variance (drug × dose × day × interval) was performed on the amount of fluid consumed. The main effect for day was significant, $F(3,48)=5.484$, $p<0.01$, which shows that fluid consumption increased over days in all groups. The main effect for interval was also significant, $F(3,48)=159.613$, $p<0.00001$, with water consumption being greatest during the first thirty minute interval. The significant three-way interaction of drug × dose × time, $F(3,48)=2.886$, $p<0.05$, indicated that after the initial thirty minute interval, drinking was similar in all groups.

EXPERIMENT 3

METHOD

The effects of a higher (1.0 mg/kg) IP dose of NAL were studied along with both an equivalent and an equimolar dose of Q-NAL. This dose of NAL has been shown to reduce fluid consumption reliably [6,21].

Animals

The rats from Experiment 2 were used in this study after a 7 day interval with free access to food and water.

Drugs

Animals received either a 1.0 mg/kg dose of NAL, 1.0 mg/kg or 50.0 mg/kg dose of Q-NAL, or saline control.

Procedure

Testing was carried out in the same manner as in Experiment 2 with the exception that the animals were tested for only one day, as in most studies of suppression of fluid intake.

RESULTS

The results of the cumulative mean fluid intakes over time interval from Experiment 3 are presented in Fig. 1. A mixed analysis of variance (drug × interval) was performed on the amount of fluid consumed in Experiment 3. Both main effects were significant: for drugs, $F(3,16)=10.527$, $p<0.001$; and for interval, $F(3,48)=397.992$, $p<0.00001$. The interaction between drug and interval was also significant, $F(9,48)=12.963$, $p<0.00001$, showing once again that effects of the drugs occurred in the initial thirty min interval. Scheffe's test revealed that reliable differences exist between the control group, the dose of 1.0 mg/kg NAL and the 50.0 mg/kg dose of Q-NAL, as well as between 1.0 mg/kg dose of Q-NAL, the 1.0 mg/kg NAL and the 50.0 mg/kg dose of Q-NAL. No other comparisons were statistically significant.

GENERAL DISCUSSION

The results of these experiments are, for the most part, in agreement with previously reported findings on the actions of NAL and its Q-form [6]. Systemic injections of a low (0.01 mg/kg) dose of NAL and a balanced dose of Q-NAL were within the range of controls. Although a few studies have suggested that this low dose may actually increase drinking behavior [3, 7, 20], the effect is not always found and was not seen in this study.

Several studies have suggested that Q-forms of opiate antagonists do not cross the Blood-Brain Barrier (BBB) since peripheral injections of Q-NAL and Q-naltrexone both failed to reverse effects that are normally NAL reversible even though they were effective when given centrally [6, 11, 19, 27, 28]. In Experiment 3, however, an IP injection of 50 mg/kg of Q-NAL was found to suppress drinking. This suggests that it does cross the BBB, at least at high doses, although a peripheral action can not be ruled out. The animals, however, did not appear to be stressed on observation during Experiment 3. The lack of an effect on drinking of 1 mg/kg of Q-NAL might be due to insufficient amounts of it crossing the BBB and is in agreement with the results of previous studies using doses less than 40 mg/kg [6, 11, 19, 27, 28].

Since the binding properties of the Q-forms of antagonists have been reported to be far less potent than that of NAL [28], it was not surprising to find in Experiment 3 that, while not being significantly different, the 1 mg/kg dose of NAL produced greater suppression of drinking than the Q-NAL. The dose of 50 mg/kg Q-NAL that had been chosen to adjust for molarity and binding differences was less potent, suggesting that, although it probably crossed the BBB, it might have had more difficulty than NAL. Thus, it appears that sufficiently high doses of Q-NAL can alter behaviors such as drinking after peripheral administration.

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