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Dopaminergic Involvement in Withdrawal Hypothermia and Thermoregulatory Behavior in Morphine Dependent Rats

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COX, B., M. ARY AND P. LOMAX. *Dopaminergic involvement in withdrawal hypothermia and thermoregulatory behavior in morphine dependent rats.* PHARMAC. BIOCHEM. BEHAV. 4(3) 259–262, 1976. – Thermoregulatory behavior was assessed in the rat by measuring the time taken to escape from a radiant heat source. The time to escape and the rise in core temperature accompanying exposure to heat were greater in morphine dependent (1 × 75 mg SC pellet implant for 72 hr) than in control rats. Injection of naloxone (1 mg/kg) into dependent rats produced a withdrawal hypothermia and decreased the time taken to escape from the heat source. Since rats undergoing withdrawal avoided heat at the same time that their core temperature was falling, the hypothermia is most likely due to a downward setting of the central thermostats rather than a direct activation of heat loss pathways. Both the withdrawal hypothermia and the behavioral changes were blocked by pimozide pretreatment (0.5 mg/kg) implicating a dopaminergic mechanism in the downward setting of the thermostats. Administration of naloxone 144 hr after pellet implantation produced similar effects to those in the 72 hr implanted group. Injection of morphine sulfate (4 mg/kg) 144 hr after implantation increased both the core temperature and the time taken to escape from heat suggesting that the effect of morphine in the dependent rat is to produce an upward setting of the thermostats.

Thermoregulatory behavior Morphine dependence Naloxone withdrawal Hypothermia Dopamine Pimozide

ALTHOUGH several putative neurotransmitters have been implicated in the development of tolerance and dependence to the narcotic analgesics [14], there has been no general agreement on the precise role of any one substance. Dopamine has been reported to be involved in the mediation of withdrawal hypothermia in the morphine dependent rat [11] and this suggestion is supported by the more recent finding [4] that pimozide, a selective dopamine receptor blocking drug [1], markedly reduced withdrawal hypothermia after direct injection into the preoptic/anterior hypothalamic (PO/AH) thermoregulatory centers. Thus, not only would withdrawal hypothermia appear to be dopamine mediated, it would also appear to be central in origin and associated with structures known to be involved in the thermoregulatory process [13].

Recently a method of measuring thermoregulatory bheavior in the rat has been developed [5] which differentiates between alterations in the central thermostats and changes only in thermoregulatory effector pathways. It was decided therefore to look for changes in behavioral thermoregulation which accompany morphine dependence and naloxone induced withdrawal and to determine what role, if any, dopamine plays in such behavioral responses.

METHOD

Male Sprague Dawley rats weighing 220–320 g were housed individually and rendered dependent by subcutaneous implantation of 1 specially formulated [8] morphine pellet containing 75 mg free base. Withdrawal was precipitated by injection of naloxone hydrochloride (1.0 mg/kg IP) either 72 or 144 hr after pellet implantation.

In the temperature studies the rats were placed in restraining cages at an ambient temperature of 18°C and the rectal temperature was monitored continuously by means of a rectal thermistor probe inserted to a depth of 6 cm. In the behavioral studies a rectal probe was also used to measure temperature, but rats were only restrained for sufficient time to allow recordings to be made (usually less than 1 min).

Behavioral thermoregulation was assessed by a method described in greater detail elsewhere [5]. Briefly, the method involves determination of the time a rat takes before escaping from a radiant heat source (250 W infrared) placed 65 cm above an enclosure 80 cm long, 8 cm wide and 35 cm high. After prior conditioning to the test chamber, control or drug injections are made and the time the rat remains under the lamp is recorded. As soon as the rat moves away the lamp is switched off for a standard time period before repeating the test. The mean time for 3 trials is used as the measure of duration of exposure to the heat lamp.

Drugs for injection were prepared in sterile NaCl (0.9%) solution and injected IP (dose volume 1 ml/kg).

RESULTS

The maximum change in body temperature occurring after injection of naloxone (1 mg/kg IP) into 72 hr pellet implanted rats is shown in Table 1. Pellet implanted rats injected with saline alone showed only a small non-significant rise in rectal temperature whereas, in rats receiving naloxone (1 mg/kg IP), a significant fall in core temperature was seen. The maximum fall in temperature usually occurred between 30 and 40 min after the injection of naloxone. Pretreatment with pimozide (0.5 mg/kg IP 2 hr before) had no significant effect on the rectal temperature of rats. Pimozide pretreatment significantly antagonized the naloxone induced fall in body temperature of morphine dependent rats.

Thermoregulatory behavior in pellet implanted rats is shown in Table 2. Control rats (Group 1) which had received no pellet implant had a mean time of exposure to the heat lamp of 6 min. This was significantly increased in the pellet implanted rats (Group 2) to more than 12 min. When pellet implanted rats were injected with naloxone (1.0 mg/kg IP) 10 min prior to commencement of behavioral testing (Group 3) their time before escape from the heat lamp was significantly less than that of the non-withdrawn dependent rats and was also within the control range. Pimozide pretreatment (Group 4) prevented this naloxone induced decrease, while not significantly modifying the exposure time of the non-withdrawn pellet implanted rats (Group 5).

All rats receiving an injection of naloxone, including the pimozide pretreated group, showed typical withdrawal signs, such as teeth chatter, wet dog shakes, chewing and writhing. These signs were particularly evident during the first 15 min after naloxone injection.

When 144 hr pellet implanted rats (Group 6) were tested, the mean time before escape from the heat lamp was significantly less than that in the 72 hr pellet implanted group (Group 2) but was not quite as low as that of the control or naloxone withdrawn group. Injection of naloxone into the 144 hr pellet implanted rats (Group 7) produced a further decrease in time before escape from the heat lamp to a value well within the control range. Morphine sulfate (4 mg/kg) injected into 144 hr pellet implanted rats (Group 8) increased their duration of stay under the heat lamp to a value not significantly different from the 72 hr implanted group. All rats in the 144 hr implanted group receiving naloxone showed typical withdrawal behavioral signs.

Changes in body temperature occurring during the thermoregulatory behavior experiment are shown in Table 3. None of the drug treated groups had initial rectal temperatures significantly different from those of the saline controls (Group 1). During the behavioral experiment these saline controls allowed their rectal temperature to rise by 0.3°C–38.1°C before escaping from the heat lamp. Rats implanted with a morphine pellet for 72 hr (Group 2) allowed their rectal temperature to rise by 1.2°C, an increase significantly greater than that in the control group. After naloxone injection (Group 3) the rectal temperature of the pellet implanted group increased by only 0.4°C, an increase not significantly different from that in control rats, but significantly less than that in the pellet implanted group. Pimozide pretreatment (Group 4) prevented the effect of naloxone and the rats allowed their temperature to rise by 1.9°C–39.5°C before escaping from the heat source. A similar increase in temperature was seen in implanted rats receiving pimozide alone (Group 5). Rats receiving pellet implants 144 hr previously allowed their temperatures to rise by 0.8°C during the behavioral test (Group 6). After naloxone injection (Group 7) this effect was antagonised and no increase in temperature was observed during the experiment. Morphine sulfate (4 mg/kg) injected into 144 hr pellet implanted rats (Group 8) changed the response of the group so that the core temperature increased by 0.4°C during the 30 min period before the behavioral test. During the behavioral test itself these rats allowed their rectal temperature to rise by 1.6°C–39.4°C before escaping from the heat source.

TABLE 1

MEAN MAXIMUM CHANGE IN RECTAL TEMPERATURE AFTER NALOXONE (1 MG/KG) INJECTION INTO MORPHINE DEPENDENT* RATS INJECTED EITHER WITH SALINE OR PIMOZIDE (0.5 MG/KG IP).

Drug	Dose (mg/kg)	Number of rats	Mean maximum change in rectal temperature† (°C±SE)
Saline	—	5	+0.02±0.22
Naloxone	(1.0)	14	−1.37±0.14‡
Pimozide	(0.5)	15	−0.05±0.17§
Naloxone + Pimozide	(1.0) + (0.5)	17	−0.87±0.21‡§

*SC implant of 1 x 75 mg morphine pellet 72 hr previously.
†Significantly different from ‡saline control; §naloxone (1 mg/kg); $p<0.05$ Mann-Whitney U test.

TABLE 2

THERMOREGULATORY BEHAVIOR IN MORPHINE DEPENDENT RATS

Group	Time after pellet implant (hr)	Drug (mg/kg)	Pretreatment time (min)	Mean time to escape from heat lamp* (min±SE)	N
1	0	Saline	10	6.0±0.51	6
2	72	Saline	10	12.6±1.9†	5
3	72	Naloxone (1.0)	10	6.4±1.6‡	5
4	72	Pimozide (0.5) + Naloxone (1.0)	120 10	12.5±2.1†§	6
5	72	Pimozide (0.5)	120	16.9±2.3	5
6	144	Saline	10	7.7±1.2	5
7	144	Naloxone (1.0)	10	6.4±1.9‡	5
8	144	Morphine (4)	30	12.7±0.8†#	5

*Significantly different from †Group 1; ‡Group 2; §Group 3; #Group 6; $p<0.05$ Mann-Whitney U test.

DISCUSSION

Drugs which activate dopaminergic systems have consistently been shown to produce hypothermia in rodents

TABLE 3

MEAN RECTAL TEMPERATURES OF RATS UNDERGOING THERMOREGULATORY BEHAVIOR EXPERIMENT

Group	Time after pellet implant (hr)	Drug (mg/kg)	Pretreatment time (min)	Mean Rectal Temperature (°C ± SE) Initial	Final*
1	0	Saline	10	37.8±0.2	38.1±0.3
2	72	Saline	10	37.9±0.2	39.1±0.2†‡
3	72	Naloxone (1.0)	10	37.5±0.2	37.9±0.5
4	72	Pimozide (0.5) + Naloxone (1.0)	120	37.8±0.2	39.5±0.5‡
5	72	Pimozide (0.5)	120	37.6±0.2	39.5±0.2
6	144	Saline	10	38.1±0.2	38.9±0.4
7	144	Naloxone (1.0)	10	38.2±0.2	38.2±0.6
8	144	Morphine (4.0)	30	37.8±0.1	39.4±0.3§

*Measured immediately after escape from heat lamp.
Change in temperature significantly different from †Group 1; ‡Group 3; §Group 6; $p<0.05$ Mann-Whitney U test.
Number of rats in each group is shown in Table 2.

after either systemic [2, 6, 7] or intracerebroventricular injection [10]. Therefore it seemed reasonable to determine what part, if any, dopamine played in withdrawal hypothermia in the rat. That pimozide, a selective dopamine receptor blocker [1], reduced the withdrawal hypothermia suggested that dopamine was indeed involved. Hypothermia may be due either to a downward setting of the central thermostats or due to a direct activation of heat loss pathways. As the rats escaped earlier from the heat source after naloxone, even though their core temperature was falling, this strongly suggests an action involving the central thermostats. This view is consistent with the previous report [4] that pimozide reduces withdrawal hypothermia after injection into the PO/AH thermoregulatory centers [9]. Testing of thermoregulatory behavior was delayed until 10 min after naloxone injection because, although core temperature was still falling at this time, the peak of the other withdrawal signs had passed. This therefore minimized the risk of other behavioral responses interfering with the thermoregulatory test. The dependent rats given pimozide apparently had a longer stay under the heat lamp than those given saline but the difference did not achieve the accepted ($p<0.05$) level of statistical significance. If the increased heat exposure in this group was meaningful then it could be due to either a dopamine receptor blocking action or to some other nonspecific effect.

In parallel with its effect on the naloxone-induced hypothermia, pimozide also blocked the changes in thermoregulatory behavior, suggesting that the withdrawal hypothermia is mediated via a dopaminergic mechanism and that dopamine is acting to alter the set point. Pimozide does not decrease all the features of withdrawal. Some are unaffected and others, such as chewing, head shakes and writhing, are increased [4]. Therefore the modification of withdrawal thermoregulatory behavior cannot be simply explained by depression of other withdrawal signs.

Rats made dependent on morphine stayed longer under the heat lamp than the saline controls. As these rats were truly dependent on morphine and showed no catatonia or reduced responsiveness to other stimuli (handling, tail pinch etc.), then this increased exposure to heat was unlikely the result of a non-specific lack of responsiveness. If there is a downward setting of a thermostat in withdrawal, it seemed possible that there might be an upward setting during dependence. The upward setting of the thermostat could explain why dependent rats remained longer under the heat lamp than controls permitting their core temperature to increase by more than 1°C. Previously it has been shown that, on repeated injection, an initial hypothermic dose of morphine causes hyperthermia [12]. The present study demonstates a morphine hyperthermia in 144 hr morphine dependent rats which was accompanied by an increased stay under the heat lamp. This finding is consistent with the hypothesis that morphine acts to raise the set point in the dependent rat.

One possible complication in the interpretation of the behavioral studies was that the analgesic drugs could be altering perception of heat stimuli rather than set-point. This possibility would seem unlikely because other workers have shown [3] that, 3 days after pellet implantation, rats gave a response time on the hot plate test which was identical to controls, even after morphine (15 mg/kg SC) injection. Thus at the time we measured thermoregulatory behavior perception of a heat stimulus was apparently normal. Further, we have shown (unpublished observations) that a low, hyperthermic, dose of morphine sulfate (4 mg/kg IP) in control rats only changed thermoregulatory behavior whilst the core temperature was rising. At the time of peak effect (presumed new, stable, thermostat setting) thermoregulatory behavior was normal. Finally when 6 day implanted rats were given morphine sulfate (4 mg/kg IP) their time of exposure to the heat lamp increased (Table 2) even though tolerance to the anlagesic effects persist for this time [3]. A different reason for the decreased stay under the heat lamp during naloxone-induced withdrawal must also be considered. During withdrawal rats show hyperirritability or exaggerated responses to noxious stimuli and this could possibly interfere with the behavioral test. However the conditions of the test were chosen so that

the rats received a gradual heat load rather than a noxious stimulus [5]. Further if hyperirritability was a factor the rats would be expected to respond to the heat even more quickly than controls. This was not the case; thermoregulatory behavior changed only to control levels. Therefore alterations in central controlling systems rather than peripheral perception systems would appear to be the most likely explanation for changes in thermoregulatory behavior.

In summary this study suggests that during morphine dependence rats behave as if there were an upward setting of their central thermostats. During naloxone precipitated withdrawal there is a dopamine mediated hypothermia apparently due to a downward setting of the thermostats.

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