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A Temporal Analysis of Naloxone's Suppressant Effect on Drinking

STEPHEN M. SIVIY,[1] DANIEL J. CALCAGNETTI AND LARRY D. REID

Department of Psychology Rensselaer Polytechnic Institute, Troy, NY 12181

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SIVIY, S. M., D. J. CALCAGNETTI AND L. D. REID. *A temporal analysis of naloxone's suppressant effect on drinking.* PHARMAC. BIOCHEM. BEHAV. 16(1) 173–175, 1982.—Licking activity was monitored in water deprived rats following various doses of naloxone. It was found that naloxone, at doses ranging from 0.01 to 10.0 mg/kg, had very little effect on initial drinking. However, naloxone did slow sustained drinking after 2 to 6 min into the bout, dependent upon dose. We take these results to mean that naloxone is interfering with those processes which serve to sustain continued drinking throughout a drinking bout.

Naloxone Endorphins Drinking

NALOXONE (NX), the prototypic opiate antagonist, reliably modifies the amount of water consumed by rodents. When NX is given to water-deprived rats, prior to their daily opportunity to drink, their intake is decreased by 25% to 50% [1, 2, 4, 5, 6, 7, 9, 10, 11, 15]. This effect is dose related [1, 2, 6, 7, 9], stereoselective [3,11], and occurs with other opiate antagonists [3, 9, 11]. As a result of these and other findings, a role for an endogenous opioid system in the regulation of drinking has been postulated.

An interesting aspect of NX's suppressant effect on deprivation-induced drinking is that animals, while under the influence of NX, appear (on gross inspection) indistinguishable from animals receiving saline. For example, NX does not affect an animal's latency to begin drinking [5] and they appear to drink with the same initial zeal as their saline counterparts ([5,] and observations in our lab).

A recent study [5] examined licking activity across a 30-min period at 6 min intervals, with and without NX. It was concluded that NX had its greatest effects during the initial 6 min of the bout and leads to the inference that only initial drinking is modified by NX. However, a more detailed analysis yields additional information as to NX's suppressant effect on drinking. The present study, therefore, measured intake every minute of a drinking bout.

METHOD

The subjects were eight, male, Sprague-Dawley derived rats (Taconic Farms, Germantown, NY) weighing from 250 to 350 g at the beginning of testing. The animals were individually housed in a colony room maintained at 26°C on a 12 hr light/dark cycle (lights on from 2200 hr to 1000 hr). All testing was conducted approximately 2 hr into the dark phase of the cycle. Rats had food always available and water available as specified by the procedure.

The animals were placed on a 23.80-hr deprivation schedule with a 12-min daily opportunity to drink. Twelve min was chosen in light of the pilot data collected for this experiment, where it was found that very little drinking occurred after 12 min in either NX or saline treated subjects. Water bottles, equipped with stainless steel sipping tubes, were weighed before and after each opportunity to drink to determine the amount consumed across the 12 min. Licking at the water spout was monitored by means of a drinkometer circuit and an event counter. The number of licks were recorded for each min across the 12-min period.

Once the animals adapted to this schedule (7 days), the dosing regimen began. Five doses of naloxone HCl were used (0.0, 0.01, 0.1, 1.0, 10.0 mg/kg). Injections were 15 min prior to the opportunity to drink and were given subcutaneously in a volume of 1 ml/kg. Injections were given on every third day until every subject received every dose. Half of the subjects were given their respective injections in an order of increasing dose while the remaining half were given their injections in an order of decreasing dose. The licking data were converted to ml consumed in each minute by first determining a volume/lick value for each animal during each session [total amount consumed (ml)/total number of licks] and then multiplying this value by the number of recorded licks for each min.

RESULTS AND DISCUSSION

A 2 by 5 ANOVA performed on the amount consumed between the increasing and decreasing groups across the five doses of NX showed that the order of dosing was not a

[1]Send reprint requests to Stephen M. Siviy, Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403.

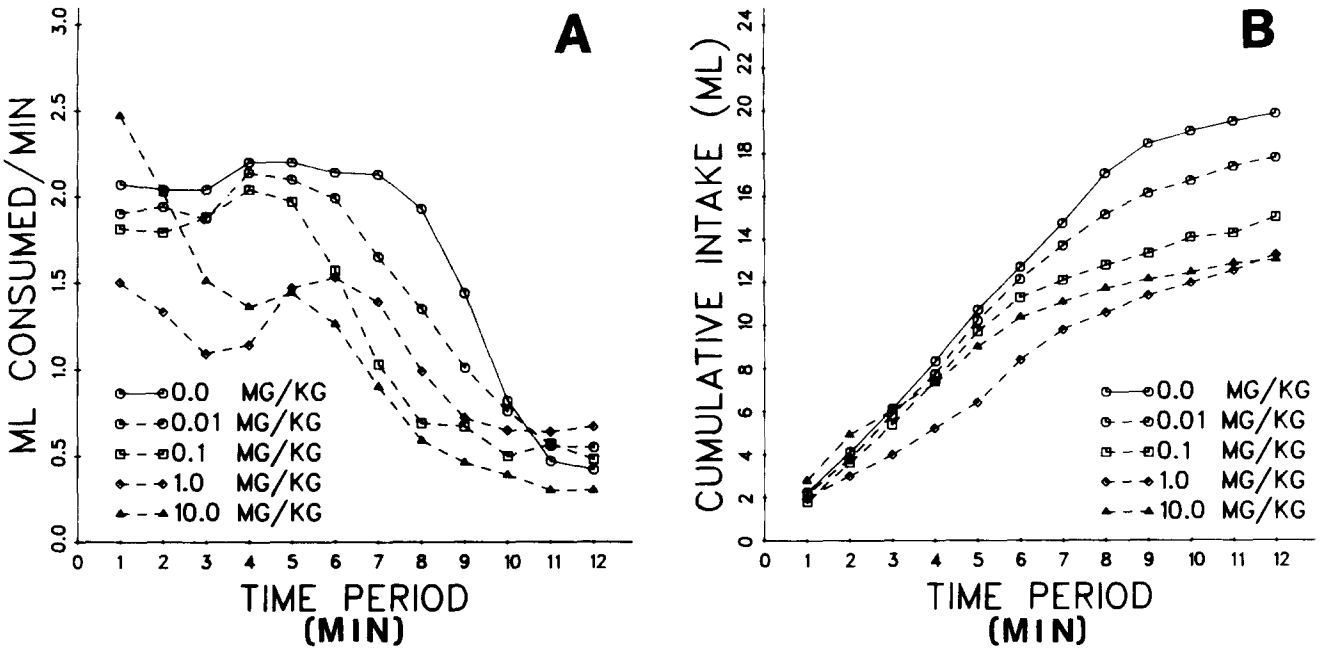

FIG. 1. The effects of various doses of naloxone on amount consumed across a 12-min drinking bout are depicted. Figure 1A depicts the running averages of amount consumed (ml) during each minute of the drinking bout. Running averages were calculated for each minute by averaging the amount consumed during that minute with the amount consumed during the minutes immediately preceding and following it. Figure 1B depicts cumulative intake (in ml) across the drinking bout.

reliable source of variance. Therefore, the drinking scores for the two groups were collapsed for further analysis.

The results of the temporal analysis are depicted in Fig. 1. Figure 1A shows the running averages of amount consumed/min across the 12 min drinking period. The data of ml consumed/min, from which the running averages were computed, conformed to a 5 by 12 factorial design, having repeated measures, with 5 levels of drug treatment and 12 factorial levels of time. An ANOVA of these data yielded a reliable effect due to doses, $F(4,28)=9.50$, $p<0.001$; a reliable effect across the 12 periods, $F(11,77)=9.06$, $p<0.001$; but no reliable interaction, $F(44,308)=1.25$.

By examining cumulative intake across the bout (Fig. 1B), a clearer picture emerges. (A similar repeated measures ANOVA performed on the cumulative intakes resulted in the same reliable main effects, as well as a reliable interaction, $p<0.001$). As can be seen, intake increases steadily in the saline-treated animals up to about 9 min, at which point there is little additional intake during the remainder of the bout. Following a dose of 0.01 mg/kg NX, intake is almost identical to that of saline controls up to about the 6- and 8-min periods. At about this time, the lines diverge and further drinking is slowed in the NX- treated animals. This same pattern is seen following a dose of 0.1 mg/kg, except that further drinking is slowed to a greater extent. At 1.0 mg/kg, intake diverges from the saline-control values after 2 min and continues at a slower rate for the remainder of the period.

When looking at cumulative intake following 10.0 mg/kg, the pattern is similar to that obtained with 0.01 and 0.1 mg/kg, except that the rate of further intake is somewhat slower. Although the amount of reduction is comparable following doses of 10.0 and 1.0 mg/kg, the patterns of intake do appear to differ. Surprisingly, the running averages for the highest dose indicate a slight facilitation of intake during the first min of the bout. Although this facilitation is slight and unreliable, we have detected similar facilitations in pilot work among animals drinking sucrose solutions (unpublished observations). NX may have non-specific effects at higher doses [14], and what we may be seeing here is a display of some of these non-opiate effects, or it may be an effect attributable to frustrative non-reward. Nevertheless, the general description of how NX leads to reduced intake is clear. Rats under the influence of NX drink as fast and steadily as controls during the initial minutes of a drinking bout, while the rate of drinking during the remainder of a bout is reduced.

It is clear from these data that NX does not affect initial drinking in the water-deprived rat. The additional detail obtained in the present study does lead to the suggestion that NX may be interfering with those processes which serve to maintain continued drinking when there is an apparent need. Along these lines, it has been suggested [13] that a major factor in the maintenance of drinking is the rewarding value associated with water when an ainmal is deprived.

NX has been shown to reduce intake of a palatable sucrose solution [16], abolish the preference for saccharin over water [8], and increase the aversity to quinine adultered solutions [8]. Furthermore, when animals are allowed to sham drink their daily ration of water, thus allowing only oropharyngeal stimulation, NX still reduces intake [12]. In conclusion, it seems highly likely that NX is interfering with those processes which serve to maintain a normal drinking bout and that this is accomplished by interfering with some aspects of the affective consequences of normal oropharyngeal stimulation.

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