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BRIEF COMMUNICATION

Effects of Pretreatment With Naloxone on Behaviours Induced by a Small Dose of Apomorphine

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SZECHTMAN, H. *Effects of pretreatment with naloxone on behaviours induced by a small dose of apomorphine.* PHARMACOL BIOCHEM BEHAV 24(6) 1779–1783, 1986.—The opiate antagonist, naloxone, was used to determine whether endogenous opioids modulate behavioural effects induced by a low dose of apomorphine. Before administering apomorphine (0.075 mg/kg) or saline, rats were pretreated with naloxone (1 mg/kg) or saline. Each subject received all 4 possible treatments (saline-saline, saline-apomorphine, naloxone-saline, and naloxone-apomorphine) in random order. Naloxone (1) reduced the frequency and (2) altered the timing of apomorphine-induced yawning, (3) reduced the frequency of apomorphine-induced stretching, (4) potentiated the effect of apomorphine on delaying grooming of the body, and (5) did not affect the hypoactivity induced by apomorphine. Moreover, like apomorphine, naloxone itself reduced activity. Furthermore, naloxone and apomorphine injected together increased the latency to groom the face. These results suggest that in some circuits, endogenous opioids interact with dopaminergic autoregulatory mechanisms.

Apomorphine Dopamine autoreceptors Grooming Hypoactivity Naloxone Opiates Rats Sedation Stretching Yawning

WHILE the discovery of opiate receptors in regions containing dopaminergic innervation [1, 25, 26, 37] illuminates the possible anatomical basis for the often observed interaction between opiate and dopaminergic systems, it does not explain how opiates produce behavioural effects that sometimes resemble activation of dopaminergic systems (e.g., [25,31]) and at other times, antagonism (e.g., [8,9]). However, it has been suggested that it may be possible to do so [16] by taking into account that dopamine systems exhibit autoregulation [15]. That is, increased neural firing, which releases dopamine, leads not only to the activation of postsynaptic neurons, but also to activation of compensatory mechanisms that inhibit dopaminergic activity. These inhibitory mechanisms include neural feedback loops [11] as well as dopamine 'autoreceptors' located on the dopamine neuron itself [5, 6, 28, 29]. Thus, depending on whether opiates (endogenous or exogenous) interact with the dopaminergic output circuits, or the dopaminergic autoregulatory mechanisms, or both, either inhibitory or excitatory behavioural effects may predominate.

Indeed, a number of studies point to an inhibitory effect of opiates on dopaminergic output neurons [19,27]. However, only a few behavioural experiments have addressed the issue of interaction between opiates and dopaminergic autoregulatory mechanisms. Among these is the study by Hernandez *et al.* [16] showing that morphine may mimic the cue provided by a low dose of apomorphine, a drug that in low concentrations (0.1 mg/kg or less) is an agonist of dopamine autoreceptors [32].

The purpose of the present study was to provide further evidence for a possible interaction between endogenous opioid systems and dopaminergic autoregulatory mechanisms. The approach used involves examining whether blockage of opiate receptors with naloxone alters the behavioural effects induced by a low dose of apomorphine. It has been suggested previously that these behavioural effects, which include reduction in activity [9,32], as well as yawning and stretching [12, 17, 23, 38] result from stimulation of dopaminergic autoreceptors.

METHOD

Animals

Subjects used in this study were 8 male Charles River Sprague-Dawley rats (440–550 g), housed 2 per cage in a colony with light on from 0700 to 1900 hr. They were well habituated to the testing environment and drug injections because of an earlier study [33] in which they experienced 7 injections of apomorphine (0.15–1.25 mg/kg) in the same apparatus as used presently.

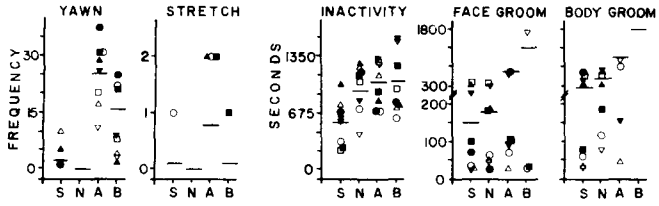

FIG. 1. Effect of pretreatment with naloxone on behaviours induced by a small dose of apomorphine. From left to right: frequency of yawns, frequency of stretching, duration of inactivity, latency to groom the face and latency to groom the body. A symbol represents the same individual rat throughout the figure. Whenever the graph indicates less than eight symbols (the total number of animals) the missing values are 0 for number of yawns, and stretches, and 1800 sec for latency to face groom, and body groom. The mean of the treatment is indicated by the horizontal line. Saline-saline (S), naloxone-saline (N), saline-apomorphine (A), naloxone-apomorphine (B).

Drugs

Naloxone hydrochloride was dissolved in saline (1 mg/ml) and apomorphine hydrochloride in saline (0.15 mg/ml) plus 0.1% ascorbic acid. Naloxone (1 mg/kg) was injected intraperitoneally and apomorphine (0.075 mg/kg) was injected subcutaneously under the scruff of the neck.

Apparatus

Each rat was tested in a low plastic dome, 9 cm high and 27 cm in diameter, which rested on a glass floor. A mirror, inclined 45° to it, permitted a bottom view of the rat's behaviour which was recorded continuously on a video cassette recorder interfaced with a time-code generator. Measurements were taken during playback of video records.

Procedure

Twenty minutes before the start of testing, the rat was put into a holding bowl for adaptation. Five minutes before testing, it was injected with naloxone (1 mg/kg) or saline. Immediately before being transferred to the testing apparatus, it received an injection of apomorphine (0.075 mg/kg) or saline. Each rat received all 4 possible treatments (saline-saline, saline-apomorphine, naloxone-saline, and naloxone-apomorphine) in random order. Tests were spaced 2–3 days apart, were 30 min in duration, and were conducted during light-on hours.

The following 5 categories of the rat's behaviour were measured: (1) *Yawning*: number of times the rat opened its mouth wide and the time of occurrence of each yawn were noted. (2) *Stretching*: number of times the rat (a) extended a forelimb to form a pillar-like structure, sometimes lifting the stretched out limb off the ground, moving it on the shoulder, and maximally abducting the digits of the paw; or (b) lifted a hindlimb off the ground spreading out the toes in the air; or (c) did both of the above. (3) *Inactivity*: duration of time the rat visibly did not move any segment of its body. The durations of the absence of movement were measured rather than of the presence of movement because inactivity was more prominent in drugged rats and was easier to score from video records. Using this scoring procedure, activity equals the duraton of test (i.e., 1800 sec) minus the duration of inactivity. (4) *Face Grooming*: and (5) *Body Grooming*: the latency

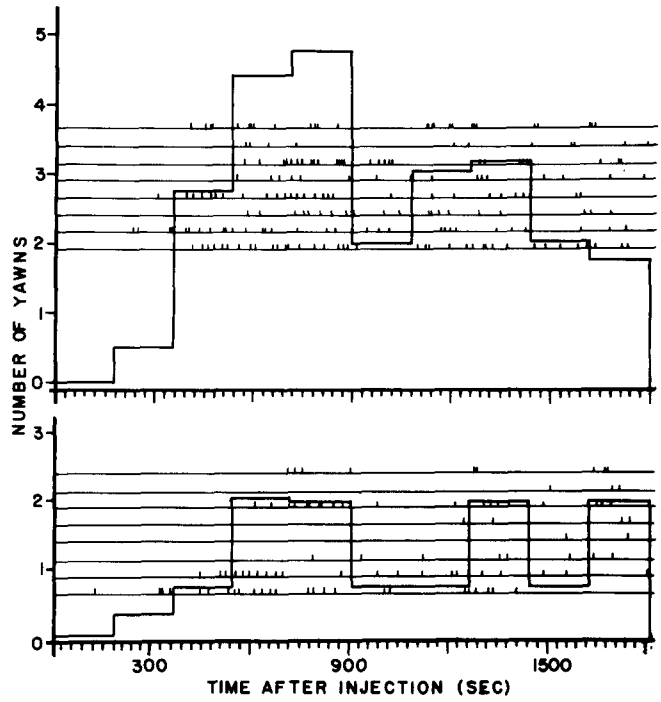

FIG. 2. Yawning following administration of saline-apomorphine (top) and naloxone-apomorphine (bottom). Each horizontal line is an individual rat; a tic represents a yawn at the indicated time. Heavy bars illustrate mean frequency of yawns of all rats during 3 min intervals.

to the first grooming of face and body, respectively. If a rat did not groom, it was given a latency of 1800 sec (the duration of the test).

Statistical Analysis

Unless noted otherwise, data were analyzed by one factor analyses of variance for repeated measures; comparisons among groups were made using Duncan's multiple range test.

RESULTS

Compared to the control (saline-saline) condition, apomorphine produced a significant change in 4 of the 5 behavioural categories examined (Fig. 1): it increased the frequency of yawning ($p<0.01$), and stretching ($p<0.05$), the duration of inactivity ($p<0.01$) and delayed the onset of body grooming ($p<0.01$). Latency to groom the face did not differ from the control condition.

Pretreatment with the opiate receptor blocker, naloxone, affected the behavioural changes induced by apomorphine as follows (Fig. 1): (1) it reduced the frequency of yawning ($p<0.01$) but not to the control mean (saline-saline vs. naloxone-apomorphine, $p<0.01$); (2) it reduced the amount of stretching ($p<0.05$) to a value that did not differ from control ($p>0.05$); (3) it did not alter the increase in inactivity ($p>0.05$); and (4) it produced an even greater delay in the onset of body grooming ($p<0.01$). In addition, naloxone and apomorphine injected together increased the latency to groom the face ($p<0.01$).

The only statistically significant effect of injecting

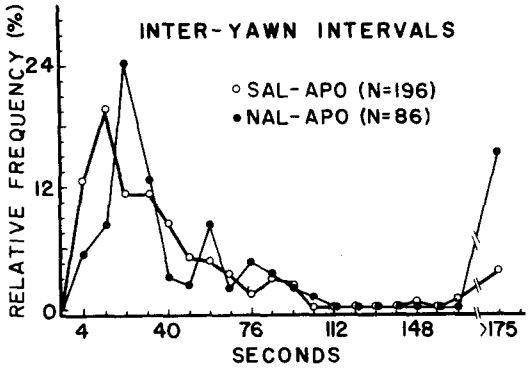

FIG. 3. Frequency distribution of inter-yawn intervals following administration of saline-apomorphine (SAL-APO) and naloxone-apomorphine (NAL-APO). N is the number of inter-yawn intervals.

naloxone-saline was an increase in inactivity compared to the control condition ($p<0.05$; Fig. 1).

Figure 2 and Fig. 3 illustrate the temporal characteristics of yawning induced by apomorphine and their alteration by pretreatment with naloxone. Four points are especially noteworthy:

First, inspection of data for each individual subject suggests that yawning came in fits; i.e., there were several yawns with relatively short inter-yawn intervals followed by a relatively long period of quiescence and then another series of yawns (Fig. 2).

Second, some yawns followed each other almost immediately, with inter-yawn intervals as short as 5 sec (Fig. 2 and Fig. 3).

Third, aside from reducing the frequency of yawns, pretreatment with naloxone altered the distribution of inter-yawn intervals, reducing the relative frequency of shorter inter-yawn intervals (Fig. 3; $D=0.187$; $p<0.05$, Kolmogorov-Smirnov test).

Finally, although pretreatment with naloxone delayed the onset of yawning [latency to first yawn (means±SEM) for saline-apomorphine was 456±48 sec vs. 867±181 for naloxone-apomorphine, $p<0.01$, paired t-test], their time course appeared similar with both treatments, exhibiting a peak at approximately 12 min after injection of apomorphine (Fig. 2)

To examine possible links between the 5 dependent measures used in this study, correlation coefficients were calculated. The only statistically significant correlation was between the frequency of yawns and duration of inactivity for the saline-apomorphine treatment ($r=-0.783$; $p<0.05$).

DISCUSSION

Present results show that pretreatment with the opiate receptors blocker, naloxone, attenuates apomorphine-induced yawning, and stretching, but does not alter hypoactivity. To the extent that apomorphine may induce these behavioural effects by stimulating dopamine autoreceptors [12, 17, 23, 38], the findings suggest that at least in some circuits, opiates interact with dopaminergic autoregulatory mechanisms [7, 10, 14, 16, 19]. Furthermore, since naloxone reduces also the relative frequency of short inter-yawn intervals, this interaction involves not only the probability of responding but also the modulation of the timing of yawns.

As both apomorphine and naloxone reduce activity, it is not possible to ascertain from the present data whether there is an opiate-dopamine interaction in the induction of hypoactivity by apomorphine.

The finding that naloxone potentiates apomorphine-induced delay in the onset of bodily grooming suggests an opiate-dopamine interaction as well. However, while the observation that a low dose of apomorphine delays the onset of grooming is a novel finding, this effect is probably mediated by receptors different than those mediating yawning, stretching, or hypoactivity. Previous research had indicated that high (post-synaptic) doses of apomorphine delay the onset of grooming [35]. Therefore, only a monophasic effect of apomorphine on the inhibition of grooming is present, a phenomenon similar to apomorphine's effect on the release of prolactin [1,36].

In addition to providing evidence for opiate-dopamine interaction, the present results reveal that yawning induced by apomorphine not only seems to have unique temporal characteristics, but also that they are modified by pretreatment with naloxone. These observations raise the possibility that the temporal characteristics of yawning may be unique in different pathological states. This suggestion is reinforced by the observation of Lehmann [21] who noted that schizophrenics are less likely to yawn but that "if yawning occurred in schizophrenic patients, it usually appeared in a peculiar, short, superficial manner and was not repeated, while the yawning of a patient with structural brain lesion tended to be frequent, deep, and prolonged."

Selective inbreeding can result in high frequencies of yawning in rats [18]. However, since the rats used in the present study came from a randomly bred population, genetic inbreeding cannot account for the much higher incidence of yawns found here than reported in the literature (25 in 30 min vs. 14 in 25 min after 0.05 mg/kg of apomorphine [29], 10 in 20 min after 0.08 mg/kg [4], or 17 in 60 min after 0.1 mg/kg of apomorphine [12]). The three most likely reasons for this discrepancy are: (1) Considering that yawning comes in fits, with some yawns following each other at 5–10 sec intervals, previous studies may have underestimated their true frequency because the rats' behaviour was monitored in real time and not on videotape. (2) The frequency of yawns in control rats increases and reaches a plateau after several exposures to the testing apparatus [20]. While in the present study subjects were well habituated to the testing cage, and therefore very likely to exhibit yawning, there is no indication that animals were adapted to the test environment in other studies. (3) Since rats used here were not drug naive, previous exposure to high doses of apomorphine may induce increased sensitivity to low doses of the drug. Although this explanation is methodologically possible, the literature suggests that just the opposite is more likely to happen. That is, pretreatment with a high (or low) dose of apomorphine reduces the behavioural effectiveness of a low dose of the drug [3,22] while pretreatment with a low dose does not alter responsiveness to a high one [13]. Future studies can resolve which of these alternatives is correct.

In conclusion, the present findings provide support for the hypothesis that endogenous opioid systems interact with dopaminergic autoregulatory mechanisms. Furthermore, the results show that naloxone modifies not only apomorphine-induced yawning and stretching, but also the temporal characteristics of yawning raising the possibility that this feature of the behaviour may prove useful as a marker of some pathological states.

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