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BRIEF COMMUNICATION

Effects of Naloxone on Heroin-, Amphetamine- and Caffeine-Stimulated Locomotor Activity in the Rat[1]

N. R. SWERDLOW,* F. J. VACCARINO† AND G. F. KOOB‡[2]

**Medical Scientist Training Program, School of Medicine, University of California, San Diego, CA*
†Division of Life Sciences, University of Toronto, Scarborough Campus, Scarborough, Ontario, Canada
‡Preclinical Neuroscience and Endocrinology, Department of Basic and Clinical Research
Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037

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SWERDLOW, N. R., F. J. VACCARINO AND G. F. KOOB. *Effects of naloxone on heroin-, amphetamine- and caffeine-stimulated locomotor activity in the rat.* PHARMACOL BIOCHEM BEHAV **23**(3) 499–501, 1984.—We investigated the effects of naloxone on the locomotor activating properties of heroin (0.25 mg/kg SC), d-amphetamine (0.25 mg/kg SC) and caffeine (7.5 mg/kg SC). Naloxone eliminated heroin-stimulated locomotion at doses approximately six times lower than those that blocked amphetamine-stimulated locomotion. Caffeine-induced locomotor activation was insensitive to naloxone at all doses tested. These results suggest that central opiate systems are differentially involved in the behavioral activation produced by heroin, amphetamine and caffeine.

Heroin Amphetamine Caffeine Naloxone Locomotor activity Behavior

SOME evidence suggests that blockade of central opiate receptors can alter the locomotor-activating properties of both central opiate agonists and indirect sympathomimetics. Thus, locomotor activation produced by morphine is antagonized by the opiate receptor antagonist naloxone [5]. Similarly, the locomotor-activating properties of the indirect dopamine (DA) agonist amphetamine are attenuated by opiate receptor blockage [8].

While studies utilizing intracerebral injections and selective DA lesions have implicated the nucleus accumbens (N.Acc.) as one site involved in mediating the locomotor-activating properties of both opiates [5] and amphetamine [4], the precise neural mechanisms that underlie this opiate-DA interaction remain unknown. For example, while amphetamine-stimulated locomotion is eliminated by either destruction of N.Acc. DA terminals [4] or by systemic injection of low doses of DA receptor antagonists [7], we recently found that the locomotor-activating properties of the opiate heroin are unaffected by destruction of N.Acc. DA terminals, and are attenuated by DA receptor antagonists only at cataleptic doses [14]. It therefore appears unlikely that opiate-stimulated locomotion results from DA-mediated neural substrates. Less clear, however, is how blockade of central opiate activity with naloxone attenuates amphetamine-stimulated locomotion.

The purpose of the present study was to investigate the pharmacological specificity of this naloxone action. We examined the effects of opiate receptor blockade on the locomotor-activating properties of heroin, amphetamine and caffeine, which is believed to act largely independent of central opiate systems [2]. Our results suggest that opiate receptors are directly involved in the locomotor-activating properties of heroin, and indirectly involved in the activating properties of amphetamine. In addition, we found little evidence to support an opiate involvement in caffeine-stimulated locomotion.

METHOD

Eighty-eight male albino Wistar rats (200–250 g, Charles River Laboratories) were housed in groups of three, exposed to a normal 12 hr light-dark cycle, with free access to food and water, and handled daily for five days. One day before behavioral testing, each animal was placed individually for three hours into a photocell cage, as previously described [2], in order to habituate them to the testing environment. Each cage measured 20×25×36 cm with two horizontal infrared photocell beams across the long axis 2 cm above the floor. On the following day, the rats were habituated to the cages for an additional 90 min, and then randomly divided

[1]This is publication number *3677 BCR* from the Research Institute of Scripps Clinic, La Jolla, CA.
[2]Reprint requests should be addressed to G. F. Koob.

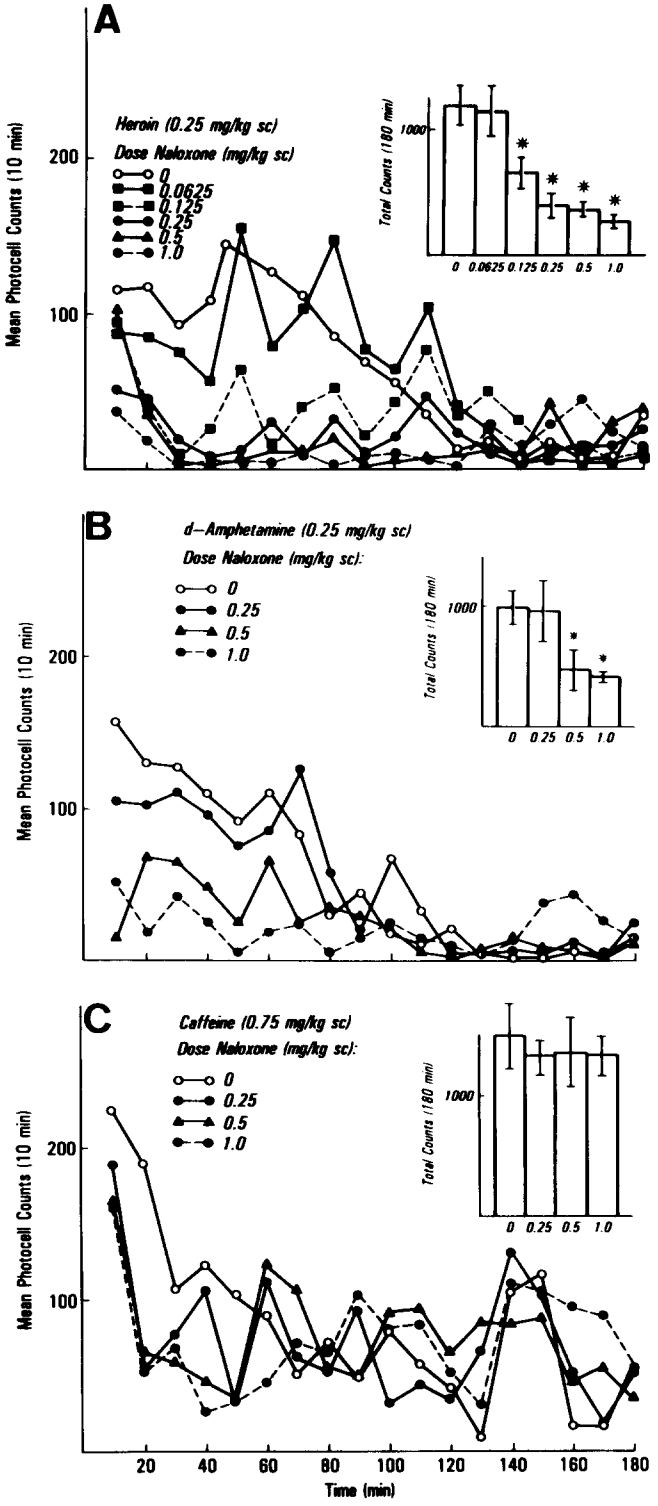

FIG. 1. Locomotor activity during 180 min test session. Following 90 min habituation period, animals were treated with naloxone (0–1.0 mg/kg SC) followed 2 min later by: (A) heroin (0.25 mg/kg SC), (B) d-amphetamine (0.25 mg/kg SC) or (C) caffeine (7.5 mg/kg SC). Insert histogram indicates total photocell counts for 180 min test period. *Refers to significant Student's t-test comparison to 0 mg/kg naloxone dose following significant ANOVA main effect.

into two groups. One group of animals was treated with one of four doses (0, 0.25, 0.5 or 1.0 mg/kg SC) of naloxone HCl. The animals were then injected 2 min later with either d-amphetamine sulfate (0.25 mg/kg SC, n=24) or caffeine (7.5 mg/kg SC, n=24), with six amphetamine- and caffeine-treated animals receiving each of the four doses of naloxone. A second group of animals was treated with one of six doses (0, 0.0625, 0.125, 0.25, 0.5 or 1.0 mg/kg SC) of naloxone HCl, with ten animals receiving 0 mg/kg naloxone, and six animals receiving each of the remaining five doses. These animals were then injected 2 min later with heroin (0.25 mg/kg SC, n=40). All injections were given in a volume of 1 ml/kg body weight, with physiological saline as the vehicle. The animals were then returned to the photocell cages, where their locomotor activity was measured for 180 min. These doses of heroin, amphetamine and caffeine were chosen since they have been found to produce approximately equal levels of locomotor activation. Comparisons of locomotor activity within each stimulant group was made using a two-way ANOVA with repeated measures on one factor, time. A significant main effect was followed by individual means comparison using a Student's t-test. The dose of naloxone which inhibited heroin, amphetamine- and caffeine-stimulated locomotion over the total 180 min period by 50% (ED50) was extrapolated from the line of best fit calculated using multiple approximations generated from an exponential equation.

RESULTS

Analysis of photocell activity in heroin-, amphetamine- and caffeine-treated animals revealed very different responses to naloxone. Heroin-stimulated locomotion (Fig. 1A) was significantly blocked by 0.125 mg/kg of naloxone (ANOVA main effect: $F(5,39)=7.52$, $p<0.001$; Student's t-test comparison of 0.125 mg/kg dose to 0 mg/kg dose: $t(14)=2.73$, $p<0.05$). Amphetamine-stimulated locomotion was attenuated by naloxone, but only at higher doses (ANOVA main effect: $F(3,23)=3.23$, $p<0.05$; Student's t-test comparisons of 0.5 mg/kg dose to 0 mg/kg dose: $t(10)=2.30$, $p<0.05$; Fig. 1B). In comparison, there was no significant main effect of naloxone on caffeine-stimulated locomotion, $F(3,23)<1$, ns, but there was a significant dose $\times$ time interaction, $F(51,408)=1.47$, $p<0.05$; analysis of simple main effects revealed that this interaction resulted from a decrease in caffeine-stimulated locomotion produced by all naloxone doses at a single time point (20 min) (Fig. 1C). The differences in sensitivity of heroin and amphetamine to naloxone are reflected in the ED50 for antagonism of stimulant-enhanced locomotion: heroin—0.12 mg/kg; amphetamine—0.67 mg/kg; no finite value for an ED50 for antagonism of caffeine could be generated from an exponential plot. Comparison of the regression lines for antagonism of heroin- and amphetamine-stimulated locomotion revealed significant differences in the y-intercepts, $t(48)=23.67$, $p<0.001$, and slopes, $t(48)=4.84$, $p<0.001$.

DISCUSSION

Our results confirm previous reports that naloxone antagonizes the locomotor-activating properties of the indirect DA agonist amphetamine [8], and also blocks the locomotor activation produced by centrally-active opiates [5]. These effects are not likely to be the result of non-specific motor depressant effects of naloxone, since the highest dose of naloxone tested produced only transient decreases in caffeine-stimulated locomotion.

The effect of naloxone on amphetamine locomotion indicates an opiate involvement in the neural substrates underlying the locomotor-activating property of this drug. One explanation for this finding, as previously described [6], might be that opiate receptors in the region of mesolimic DA terminals exert a tonic facilitatory influence on DA synthesis and release within the N.Acc. Blockade of these receptors might indirectly antagonize the locomotor-activating properties of amphetamine, which depend on the release of DA from these N.Acc. terminals [4]. It is indeed possible that an action of naloxone on DA terminals within the N.Acc. might be responsible for the observed transient attenuation of caffeine-stimulated locomotion, since a similar transient effect has been reported following destruction of N.Acc. DA terminals [11]. Such a presynaptic site for this opiate-DA interaction might account for the finding that locomotor activation resulting from direct stimulation of postsyanptic supersensitive N.Acc. DA receptors following destruction of DA terminals is not significantly decreased by doses of naloxone as high as 5 mg/kg [9].

The more potent effects of naloxone on heroin-stimulated locomotion, however, suggest a more direct opiate involvement in this behavioral activation. While the receptor responsible for this opiate-stimulated locomotion might be located either pre- or postsynaptic to mesolimbic DA terminals, the fact that enkephalin-stimulated locomotion is not antagonized by 6OHDA-induced destruction of mesolimbic DA terminals [3] argues against a presynaptic site of action. Consistent with these findings, we have recently noted a similar insensitivity of heroin-stimulated locomotion to destruction of DA terminals within the N.Acc. [14].

Thus, it may be that the opiate-DA interaction within the N.Acc. involves opiate receptors located both pre- and postsynaptic to DA terminals. The presynaptic opiate receptor might impose a weak modulatory influence on DA release, and thus account for the naloxone-amphetamine interaction reported herein. An opiate receptor postsynaptic to the DA terminal, however, might mediate the direct activating properties of opiates. Indeed, the finding that locomotor activation produced by heroin is antagonized by direct injection of methyl-naloxonium HCl into the N.Acc. [1] but not by destruction of presynaptic N.Acc. DA terminals [14] supports this hypothesis. Other work in our laboratory [9, 10, 11] indicates that N.Acc. efferent projections to the substantia innominata/lateral preoptic region (SI/LPO) form the critical link in the behavioral output of N.Acc. DA stimulation. Future studies will be directed towards determining whether this N.Acc.-SI/LPO projection is critical for the behavioral activation associated with opiates.

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