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BRIEF COMMUNICATION

Locomotor Depression in Mice by Norcocaine Does Not Involve Central α_2-Adrenergic or Presynaptic Dopamine Receptors[1]

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REITH, M E A AND A LAJTHA *Locomotor depression in mice by norcocaine does not involve central α_2-adrenergic or presynaptic dopamine receptors* PHARMACOL BIOCHEM BEHAV **24**(2) 305–307, 1986 —The inhibition of spontaneous locomotor behavior of mice by norcocaine was antagonized neither by the adrenoceptor antagonists yohimbine and phentolamine, nor by the neuroleptics haloperidol and spiperone, at low doses aimed at presynaptic dopamine receptors In contrast, the antagonists were effective in reducing the hypomotility induced by clonidine and apomorphine, respectively These results make it unlikely that central α_2-adrenergic or presynaptic dopamine receptors are involved in the hypomotive effect of norcocaine

Locomotion Norcocaine Local anesthetics Motor inhibition α-Adrenoceptors Dopamine receptors

IT is well known that cocaine can produce a remarkable increase in spontaneous locomotor behavior of mice and rats (for review see [17]) Recent studies in our lab on structure-activity relationships of this ability of cocaine showed that most cocaine congeners in fact inhibit rather than stimulate spontaneous locomotion of BALB/cBy mice Locomotor depression was observed after IP administration of norcocaine, (+)-pseudococaine, tropacocaine, or phenyltropane analogs such as WIN 35,140, WIN 35,004, and WIN 35,065-3 [13] Hypomotive effects have been reported also for α_2-adrenergic agonists such as clonidine, presumably by interactions with central α_2-adrenoceptors [5, 7, 15] We wondered whether these receptors are involved in the locomotor depression produced by cocaine congeners, since cocaine has been suggested to interact with the imidazoline recognition site of the α_2-adrenoceptor in the central nervous system [12] Thus, cocaine antagonized the inhibitory effect of the imidazoline clonidine on stimulation-evoked [^{3}H]norepinephrine release from rat cerebral cortical slices, but failed to prevent the inhibition of release by α-methylnorepinephrine [12] In the present study we investigated the possible involvement of α_2-adrenoceptors in the locomotor depression induced by IP administration of norcocaine, an important metabolite of cocaine that is formed by N-demethylation and has central activity [1, 2, 6] Since presynaptic dopamine receptors have been implicated in hypomotive effects [3, 4, 7], in addition to α_2-adrenoceptors, we also studied the effect of neuroleptics on the norcocaine-induced hypomotility

METHOD

Male BALB/cBy mice, 8–12 weeks of age, weighing 18–22 g, were used from the breeding colony of our Institute The animals were kept on a 12-hour light/dark cycle (7 a m /7 p m light), with food and water available ad lib Mice were housed in separate plastic cages (27×17×12 cm) from one to three days prior to testing in the same room in which the behavioral measurements were made Shades were drawn to reduce the light from the windows, and no artificial light was used Behavioral testing was performed between 9 a m and 5 p m and was started by placing the animal in its own home cage in an Opto-Varimex-Minor activity monitor (Columbus Instruments) and replacing the roof with a flat one without food and water After an exploratory period of 10 min, the animal was monitored for 30 min The animal was then injected with an antagonist or saline, placed back in the home cage, and monitored for 20 min Subsequently, it received an injection of agonist, norcocaine, or saline, and was monitored for another 30 min The number of activity counts

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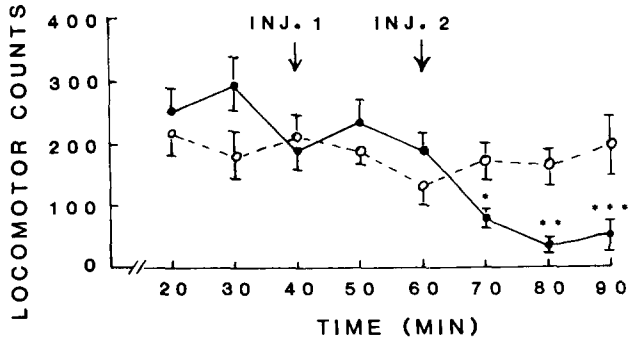

FIG 1 Effect of norcocaine on spontaneous locomotor activity of mice Locomotor counts were recorded every 10 min Injections are indicated by arrows ○— — —○, 1st injection, saline, 2nd injection, saline ●———●, 1st injection, saline, 2nd injection, norcocaine (10 mg/kg IP) Results are the mean±SEM for six animals *$p=0$ 06, **$p<0$ 01, ***$p<0$ 02, compared with control group at that time-point, two-tailed Mann-Whitney U test

produced in the last 30-min period was expressed as percent of those in the initial 30-min period This method takes into account the somewhat varied baseline levels of spontaneous activity in different animals The behavioral data presented in this paper are based on activity counts produced by the animals by interrupting consecutive infrared beams only, systematic interruptions of one beam are not included The activity counts therefore mainly represent ambulation Each animal was used only once The same drug sequences were administered at various times of the day to minimize the effect of time-related phenomena on the behavioral results

Drugs were dissolved in saline with the following exceptions Norcocaine was first dissolved in warm 25 mM sodium phosphate buffer, pH 6 7, then saline was added to obtain final concentrations of 6 2 mM sodium phosphate and 0 12 M NaCl (phosphate-saline) Haloperidol solutions were dilutions in phosphate-saline of stocks prepared by dissolving a small amount of haloperidol in one drop of glacial acetic acid and adding water, the final dilutions had a pH close to neutral Spiroperidol solutions were prepared the same way Apomorphine hydrochloride was dissolved in saline by warming All injections were given in a volume of 0 15 ml per 20 g body weight Statistical significancies, except where noted, were obtained with the two-tailed Mann-Whitney U test

RESULTS AND DISCUSSION

There was a rapid onset of locomotor depression after IP injection of 10 mg/kg norcocaine (injection 2, solid line in Fig 1) The animals spent most of the post-injection time sitting in one corner, with their backs curved and eyes open The animals were less alert but did not assume postures normally associated with sleep The hypomotive effects of norcocaine were dose related [13], and for this study we chose a dose (10 mg/kg) that causes moderate inhibition

The norcocaine-induced decrease in locomotion could not be antagonized by pretreating the animals with the α_2-adrenergic antagonist yohimbine (2 mg/kg SC) (Table 1) To assess the effectiveness of such a treatment under our conditions, we examined the interaction between yohimbine and clonidine, an α_2-adrenergic agonist In agreement with previously reported findings [5,11], pretreatment with yohimbine greatly reduced the locomotor depression induced by

TABLE 1

EFFECT OF VARIOUS ANTAGONISTS ON THE HYPOMOTILITY PRODUCED BY NORCOCAINE, CLONIDINE, AND APOMORPHINE

Drugs Injection 1 + Injection 2	Dose (mg/kg)		Motor activity % of baseline	
Saline + Saline		IP + IP	90 ± 17	(7)
Saline + Norcocaine		IP + 10 IP	25 ± 7*	(6)
Yohimbine + Norcocaine	2	SC + 10 IP	17 ± 6*	(6)
Phentolamine + Norcocaine	0 3	IP + 10 IP	17 ± 4*	(8)
Haloperidol + Norcocaine	0 1	IP + 10 IP	9 ± 2†	(6)
Spiperone + Norcocaine	0 05	IP + 10 IP	23 ± 6*	(6)
Saline + Clonidine		SC + 0 1 IP	24 ± 6*	(6)
Yohimbine + Clonidine	2	SC + 0 1 IP	66 ± 10‡	(8)
Phentolamine + Clonidine	0 3	IP + 0 1 IP	63 ± 13§	(7)
Saline + Apomorphine		IP + 0 1 SC	8 ± 3*	(6)
Haloperidol + Apomorphine	0 1	IP + 0 1 SC	30 ± 5¶	(6)
Spiperone + Apomorphine	0 05	IP + 0 1 SC	41 ± 9**	(8)
Yohimbine + Apomorphine	2	SC + 0 1 SC	10 ± 3*	(6)
Cocaine methiodide‡‡	50	IP	52 ± 12††	(9)

Results are means ± SEM's of the motility of each animal for 30 min after the second injection as percentage of its initial motility measured for 30 min prior to the first injection (baseline) The time interval between the two injections was 20 min Numbers in parentheses indicate number of animals per group *$p<0$ 004 compared with saline + saline, †$p<0$ 05 compared with saline + norcocaine, ‡$p<0$ 02 compared with saline + clonidine, §$p<0$ 05 compared with saline + clonidine, ¶$p<0$ 01 compared with saline + apomorphine, **$p<0$ 05 compared with saline + apomorphine, ††0 05 < $p<0$ 10 compared with saline + saline, two-tailed Mann-Whitney U test ‡‡This group received only one injection, after which it was monitored for 30 min

clonidine (0 1 mg/kg IP) ($p<0$ 02, Table 1) The same treatment with yohimbine was not able to partially antagonize the hypomotive effect of apomorphine (0 1 mg/kg SC), indicating that in the BALB/cBy mice used for this study α_2-adrenoceptors are less likely to be involved in the apomorphine-induced locomotor depression than in the Swiss-derived mouse strain used by Sumners *et al* [15] Another α_2-adrenergic antagonist, phentolamine (0 3 mg/kg IP), was also ineffective in protecting against the hypomotive action of norcocaine (Table 1) Yet, under the same experimental conditions, phentolamine did appreciably reduce the clonidine-induced locomotor inhibition ($p<0$ 05) There was no statistically significant effect of the α_2-adrenergic antagonists themselves on spontaneous locomotor activity, when the motilities were compared after injection of saline, phentolamine, and yohimbine, expressed as percentages of baseline activities ($p>0$ 50, test of Kruskal and Wallis)

The involvement of dopamine autoreceptors in the hypomotive action of norcocaine was assessed by pretreating animals with a low dose of haloperidol (0 1 mg/kg IP) or spiperone (0 05 mg/kg IP), aimed at selectively blocking

presynaptic dopamine receptors Neither antagonist was able to counteract the inhibition by norcocaine, haloperidol had a small but statistically significant ($p<0.05$) potentiating effect (Table 1) In contrast, both haloperidol ($p<0.01$) and spiperone ($p<0.05$) reduced the inhibitory effect on locomotion produced by apomorphine (0 1 mg/kg SC), in agreement with findings by others [3,4] There was no statistically significant effect of the antagonists themselves on locomotion ($p>0.95$, test of Kruskal and Wallis, as above for adrenergic antagonists)

The present results indicate that neither α_2-adrenoceptors nor dopamine autoreceptors in the brain are involved in the inhibitory effect of norcocaine on spontaneous locomotor behavior The effect of IP norcocaine in this study seems to be similar to that of the local anesthetic lidocaine, which produces a behavioral depression upon IV administration in rabbits and cats [16] Under our conditions, we have obtained hypomotive effects of IP administered lidocaine, and also of other local anesthetics such as tetracaine, prilocaine, procaine, and benzocaine [13] In addition, cocaine methiodide, the compound bearing a quaternary nitrogen, does not cross the blood-brain barrier, and has local anesthetic activity [14], under the present conditions, cocaine methiodide (50 mg/kg IP) appreciably inhibited locomotion (Table 1, $0.05<p<0.10$), its potency in this respect was similar to that of procaine and lidocaine (not shown) The data so far are consonant with the view that the locomotor inhibitions observed with IP norcocaine and other cocaine congeners are due to the local anesthetic potency of cocaine-related structures Although norcocaine has been reported to have central activity [1, 2, 6], we propose that upon systemic administration of norcocaine its local anesthetic potency is the dominant property The central potency of norcocaine in inhibiting neuronal uptake of dopamine in the mouse striatum is three times weaker than that of cocaine (*Biochem Pharmacol*, in press), norcocaine has been reported to be a stronger local anesthetic than cocaine [8,9] These effects may partly explain the intriguing observation that cocaine is a potent locomotor stimulant, whereas its demethylated metabolite, norcocaine, is a potent locomotor depressant The local anesthetic effect of IP norcocaine on locomotion is probably not due to factors governing its entry into and clearance from the brain, since peripheral administration of [^{3}H]norcocaine to rats has been shown to result in brain levels of norcocaine somewhat higher than those of cocaine after peripheral administration of [^{3}H]cocaine, whereas there was little difference between the brain-to-plasma ratios of [^{3}H]norcocaine and [^{3}H]cocaine and between their half lives in the brain [10] In addition, peripherally administered norcocaine and cocaine do share some central effects, such as a decrease in fixed-ratio food-maintained responding in rats and maintenance of self-administration behavior in monkeys [1] Quantitative differences in rats between norcocaine and cocaine have been reported for their potency in generalizing to cocaine in a discriminative stimulus paradigm [2] and in reducing food intake [1], whereas qualitative differences exist in the effects on fixed-interval food-maintained responding [1] In that same study, cocaine (10, 20, and 40 mg/kg IP) enhanced locomotor activity of Wistar rats, but the same doses of norcocaine did not Cocaine has been suggested to specifically interact with the imidazoline recognition site of the central α_2-adrenoceptor [12], and the possibility exists that this action contributes to the centrally stimulatory effect of cocaine The present results, however, make it unlikely that central α_2-adrenoceptors are involved in the inhibitory effect of norcocaine on spontaneous locomotor behavior of mice

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