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BRIEF COMMUNICATION

Pimozide Blocks the Open-Field Hyperactivity Produced by Lesions of the Ventral Noradrenergic Bundle in Rats

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MILLAN, M. H. AND M. J. MILLAN. *Pimozide blocks the open-field hyperactivity produced by lesions of the ventral noradrenergic bundle in rats.* PHARMACOL BIOCHEM BEHAV **20**(3) 473–477, 1984.—Selective, bilateral, radiofrequency destruction of the ventral noradrenergic bundle resulted in a substantial fall in hypothalamic but not cortical levels of noradrenaline. Ventral bundle-lesioned rats displayed, as compared to sham-operated animals, a pronounced enhancement of ambulation in both peripheral and central squares together with a reduction in immobility and an enhancement of rearing upon introduction into a novel open-field space. Grooming behaviour was not, in contrast, significantly modified. Pretreatment with the dopamine receptor antagonist, pimozide, strongly decreased this hyperactivity without significantly altering the behaviour of sham animals. These data suggest that the ventral bundle plays a role in the control of open-field locomotor behaviour and that this action may be exerted via an interaction with a dopaminergic network.

Noradrenaline Dopamine Pimozide Ventral bundle Open-field Locomotor activity

THE importance of catecholamines in the control of locomotor activity is well-established and recent studies have begun to differentiate between, and more precisely characterize, the roles of particular pathways. A variety of studies have identified the mesolimbic dopaminergic projection as the major dopaminergic substrate stimulating locomotion with its striato-nigral counterpart playing a minor role in this respect and primarily related to the stereotypic effects of dopamine [1, 6, 13]. Concerning brain networks of noradrenaline (NA), non-selective pharmacological manipulations have yielded conflicting data [3, 6, 8, 13]: this appears to reflect the fact that individual noradrenergic networks fulfill differing roles. Indeed, of the two major ascending bundles, the dorsal bundle (derived from the locus coeruleus [9]) may facilitate, and the ventral bundle (VB) (from medullary-pontine somata [9,12]) possibly inhibit, locomotor activity [3,8]. Interestingly, an interaction between NA and dopamine in the control of behaviour has been postulated [1] and both biochemical studies of actions of NA-manipulations upon dopamine-turnover and behavioural investigations have yielded evidence in support of such a hypothesis [2, 3, 4, 8, 12, 16].

There are, thus, indications for, firstly, an involvement of NA and dopamine in control of locomotor behaviour and, secondly, for NA-dopamine interactions. It may be hypothesized, therefore, that there may be an interrelationship between particular noradrenergic and dopaminergic systems in the regulation of locomotor behavior. The possibility of an interplay between the VB and dopaminergic transmission in the modulation of the locomotor and other behaviour of rats exposed to an open-field space was, consequently, evaluated in the present study.

METHOD

Male, Sprague-Dawley rats, maintained under a 12 hour/12 hour natural day/night cycle, and weighing 180–190 g were housed in groups of 4 and allowed 5 days adaptation to laboratory conditions prior to operation.

The VB was bilaterally destroyed by radiofrequency in rats positioned under 50 mg/kg pentobarbital anaesthesia in a David Kopf apparatus. Electrode coordinates derived from our previous studies [11] and based on the atlas of König and Klippel [7], were relative to the inter-auricular line; horizontal, +1.1 mm, vertical, +2.8 mm and lateral ±1.4 mm. For lesions, a tip temperature of 50°C was maintained for 8 sec and, for sham operations, the unactivated electrode lowered for 8 seconds to a point 1 mm above lesions coordinates.

One week post-surgery, the influence of blockade of dopamine receptors upon open-field behaviour of VB-lesioned rats was evaluated by use of the central dopamine receptor antagonist, pimozide. Pimozide shows especial specificity in this respect with strong post- and low presynaptic activity (see [4, 14, 15]). Moderate doses (0.25 mg/kg and 0.5 mg/kg) were chosen as used in previous investigations (see [4, 14, 15]) and dissolved in 0.1 M tartaric acid.

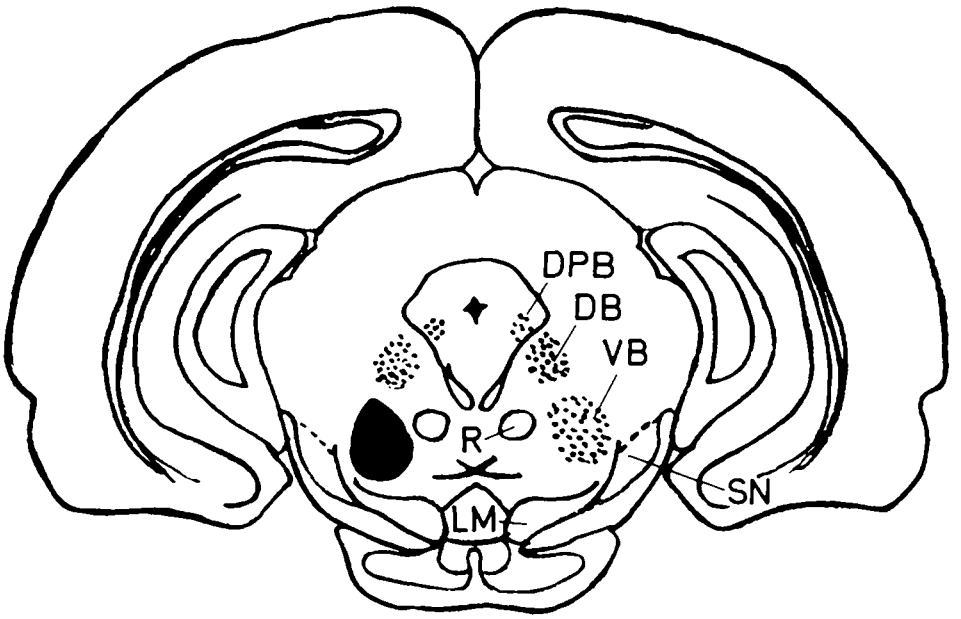

FIG. 1. Schematic illustration of position of typical lesion of the ventral noradrenergic bundle indicated by shaded area on the left. Frontal section based on the atlas of König and Klippel [7]. Abbreviations: DB (dorsal noradrenergic bundle), DPB (dorsal periventricular bundle), LM (medial lemniscus), R (red nucleus), SN (substantia nigra), VB (ventral noradrenergic bundle).

The effects of these doses were examined in separate experiments on independent groups of rats. In preliminary experiments, the solvent alone did not influence open-field behaviour. The action of pimozide was evaluated in a completely blind design. The observer was, thus, aware of neither whether a rat was lesioned or sham-operated nor whether it had received pimozide or vehicle.

The open-field apparatus, as previously used [11], consisted of a 1 m by 1 m wooden box with sides 25 cm in height, painted white and with its floor sub-divided into 25 squares, each of dimensions 20 cm by 20 cm. It was illuminated by a 60 Watt light situated 1 m above its centre. Prior to and between introduction of rats, the floor was thoroughly cleaned and 5 min allowed to pass between the testing of successive rats. Rats were gently removed from home cages, received IP injections, in a volume of 2.0 ml/kg, of either pimozide or vehicle, were returned to home cages for 1 hr, then retaken and gently placed in a corner of the open-field space. Behaviour was observed for 5 min [3,8]: the number of either central or peripheral squares completely entered by all 4 paws (ambulation), the number of rearings to a vertical position on hind feet, the time devoted to grooming and the duration of immobility, i.e., lack of any overt behaviour, was recorded.

In an independent group of rats, the influence of the α-adrenergic antagonist, phentolamine (Ciba-Geigy) dissolved in saline (5 mg/kg, 2.0 ml/kg, IP), as compared to saline, applied 30 min prior to open-field exposure was evaluated in a similar fashion.

Rats were decapitated 10 days post-operation, their brains removed, the hypothalamus and cortex dissected out and levels of NA and dopamine therein evaluated by liquid chromatography [5]. The remainder of the brain was retained for histological examinations by staining of 20 μ coronal sections with cresyl violet. In addition, the body weight and ingestive behaviour of rats was monitored after surgery.

RESULTS

The lesions were as those extensively characterized previously [11] with the zone of destruction, which was bilaterally symmetrical and consistent in shape, size and position, corresponding with the location of the VB (Fig. 1). Importantly, the lesions did not invade the dorsal bundle indicating their selectivity in eliminating the VB. A slight invasion of the substantia nigra, medial leminisus, red nucleus, and superior cerebellar peduncle was occasionally seen, but no aphagia and loss of body weight or muscular discoordination, effects which accompany damage to the above structures was observed. In the hypothalamus, the primary target of the VB [9,12]; a pronounced fall in the content of NA was seen (Table 1): residual NA is provided by the locus coeruleus, via the dorsal bundle and tegmental radiations, and by the ventral periventricular system [9]. In the cortex, in contrast, innervated by the dorsal bundle but not the VB [9], no alteration in NA levels was seen indicating that the dorsal bundle survived undamaged (Table 1). In addition, levels of dopamine were found not to be affected in either the cortex or hypothalamus (Table 1). Further, previously, we reported VB-lesions not to affect levels of serotonin in these tissues [3].

Figure 2 depicts the effect of 0.5 mg/kg pimozide upon open-field behaviour of sham and lesioned animals. VB-lesioned rats treated with vehicle displayed, as compared to vehicle-treated sham rats, a pronounced enhancement of ambulation in both central and peripheral squares, an elevation in rearing and a corresponding reduction in periods of immobility in the open-field (Fig. 2). This hyperactivity was coordinated in nature and no stereotypy was apparent. Further, time devoted to grooming, in contrast, tended to be decreased in VB-lesioned rats. Pimozide (0.5 mg/kg) abolished this VB-lesion induced hyperactivity whereas it only tended to depress the activity of sham animals (Fig. 2). Pimozide resulted, further, in a tendency to reduce grooming

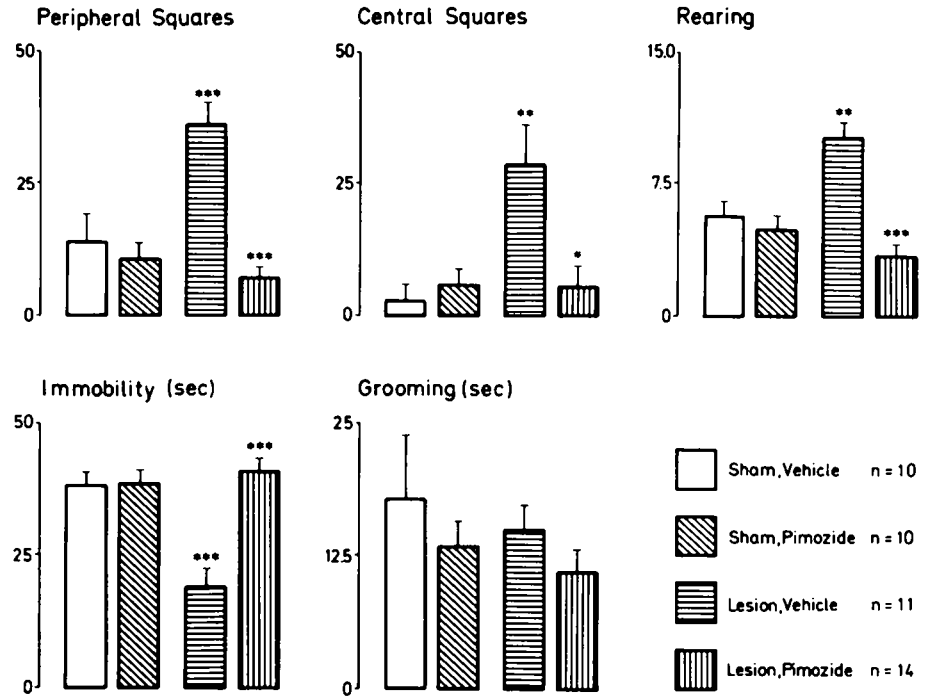

FIG. 2. The influence of pimozide (0.5 mg/kg) upon the open-field behaviour of rats subjected to lesions of the ventral noradrenergic bundle or sham operations. Data refer to number of peripheral or central squares entered, number of rearings made and time devoted to grooming or time immobile in a 5 min test. Mean±S.E.M. presented. Asterisks above lesion, vehicle columns refer to significance of differences as compared to sham, vehicle values and those above lesion, pimozide columns to significance of differences to lesion, vehicle values (Students two-tailed t-test). For ANOVA, see text. $*p<0.05$, $**p<0.01$, $***p<0.001$.

TABLE 1

	Noradrenaline (μg/g)		Dopamine (μg/g)	
	Hypothalamus	Cortex	Hypothalamus	Cortex
Sham	3.31 ± 0.11	0.23 ± 0.02	0.53 ± 0.05	0.27 ± 0.02
VB-lesion	1.55 ± 0.07*	0.22 ± 0.01	0.56 ± 0.02	0.29 ± 0.03

The influence of bilateral lesions of the ventral noradrenergic bundle (VB) upon levels of noradrenaline and dopamine in hypothalamus and cortex.
Mean ± S.E.M. indicated. N as follows: shams (20) and lesions (25).
Significance of lesion vs. sham difference indicated (Student's two-tailed t-test).
$*p<0.001$.

in both lesioned and sham rats. An analysis of variance (ANOVA) revealed a significant group-drug interaction, in each case, for peripheral squares, $F(1)=13.03, p<0.001$, central squares, $F(1)=5.68$, $p<0.02$, rearing, $F(1)=7.07$, $p<0.01$, and immobility, $F(1)=15.74$, $p<0.001$, but not for grooming, $F(1)=0.64$, $p>0.05$. These ANOVA demonstrate that pimozide is significantly more effective in blocking the activity of lesioned as compared to sham animals, whereas they do not differ as concerns its influence upon grooming. Pimozide at 0.5 mg/kg, thus, abolishes VB-lesion-evoked open-field hyperactivity. Indeed, additional studies showed that a lower dose of 0.25 mg/kg also decreased the VB-hyperactivity as indicated in Table 2. Relative to vehicle-treated VB-lesioned rats, 0.25 and 0.5 mg/kg pimozide decreased peripheral square crossings by, respectively, 45.0±4.2% and 80.3±5.9% and rearing by, respectively, 32.1±3.9% and 69.4±4.7%. Thus, 0.5 mg/kg was more effective than 0.25 mg/kg although an interpretation of dose-dependency should be made only cautiously since independent batches of rats were used for the respective doses.

Finally, as shown in Table 3, the α-noradrenergic antagonist, phentolamine (5 mg/kg) failed to influence the behaviour of either sham or lesioned rats. This indicates the selectivity of the pimozide action and that this hyperactivity does not reflect a compensatory enhanced functioning of any residual undestroyed VB fibres.

TABLE 2

		Peripheral squares	Rearing	Grooming (sec)
Sham	vehicle (8)	18.6 ± 3.1	10.1 ± 2.0	16.4 ± 4.2
	pimozide (10)	13.7 ± 3.2	7.9 ± 0.7	12.2 ± 4.7
VB-lesion	vehicle (10)	44.8 ± 4.9†	18.7 ± 1.5*	17.8 ± 3.0
	pimozide (10)	24.7 ± 4.3†	12.7 ± 1.6*	12.6 ± 4.4

The influence of pimozide (0.25 mg/kg) upon the open-field behaviour of rats subjected to either sham operations or lesions of the ventral noradrenergic bundle (VB).

Mean ± S.E.M. indicated. Significance of BV-lesion (vehicle) vs. sham (vehicle) and VB-lesion (pimozide) vs. VB-lesion (vehicle) differences indicated (Student's two-tailed t-test) *$p<0.05$, †$p<0.01$.

TABLE 3

		Peripheral squares	Rearing	Grooming (sec)
Sham	Saline (7)	14.0 ± 2.6	16.2 ± 2.9	20.9 ± 5.3
	Phentolamine (7)	12.1 ± 2.4	16.9 ± 2.3	15.8 ± 3.6
VB-lesion	Saline (7)	29.6 ± 4.3†	27.4 ± 2.1*	14.9 ± 3.2
	Phentolamine (8)	35.4 ± 4.7	25.6 ± 2.7	15.5 ± 2.7

The influence of phentolamine (5 mg/kg) upon the open-field behaviour of rats subjected to either sham operations or lesions of the ventral noradrenergic bundle (VB).

Mean ± S.E.M. indicated. Significance of VB-lesion (saline) vs. sham (saline) differences indicated. (Student's two-tailed t-test).

*$p<0.05$, †$p<0.001$.

DISCUSSION

Destruction of the VB resulted in an open-field hyperactivity in confirmation of earlier reports [11] and indicating that the VB plays a role in the control of open-field behaviour. These data are consistent with previous behavioural studies of the role of noradrenergic networks in the control of locomotor activity [2, 3, 6, 8, 13]. Pimozide attenuated this hyperactivity suggestive of its mediation via a dopaminergic network and studies of the sexual behaviour of VB-lesioned rats and their response to neuroleptics and dopaminergic agonists have, similarly, indicated that destruction of the VB may lead to a facilitation of dopaminergic transmission [2, 3, 8, 16]. The VB possibly supplies a minor input to the substantia nigra but does not project to the striatum [9,12]. Further, bilateral lesions of the VB do not alter nigral or striatal levels of dopamine [12] and VB-lesioned rats do not reveal any stereotypy. Thus, the nigro-striatal pathway is unlikely to be a mediator of VB-hyperactivity. In contrast, the VB projects to the origin and terminal fields (such as the nucleus accumbens) of the mesolimbic system, the major dopaminergic pathway for locomotor stimulation [9,12]. Lesions of the VB, indeed, deplete NA from the nucleus accumbens [12]. Further, microinjections of NA into the ventral tegmental area or accumbens produce a locomotor-depression [2,13] while VB-lesions appeared to enhance dopaminergic activity in the former (but not the latter) region [12]. These observations focus attention on the meso-limbic dopaminergic network as a possible mediator of the VB-lesion elicited hyperactivity.

The present data provide novel evidence that an interaction between noradrenergic and dopaminergic pathways is of significance in the regulation of behaviour [1]. Under stress, as comprised by the open-field [10], such an interrelationship was postulated as of particular importance [1]. Moreover, certain biochemical studies concluded that noradrenergic networks may modulate, and possibly inhibit, the activity of dopaminergic pathways [1, 4, 12].

A major criterion of a potential animal model of mania is the occurrence of a locomotor hyperactivity [17]. Pimozide possesses anti-manic properties in man [15] and an overactivity of dopaminergic transmission has been implicated as a causative factor in mania [15,17]. It may, thus, be tentatively suggested that a further characterization of VB-lesion evoked, pimozide-blockable hyperactivity might reveal a novel and instructive animal model of mania.

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