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Behavioral Effects of Neuroleptics, Apomorphine and Amphetamine after Bilateral Lesion of the Locus Coeruleus in Rats

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KOSTOWSKI, W., M. JERLICZ, A. BIDZINSKI AND M. HAUPTMANN. *Behavioral effects of neuroleptics, apomorphine and amphetamine after bilateral lesion of the locus coeruleus in rats.* PHARMAC. BIOCHEM. BEHAV. 7(4) 289–293, 1977. – Bilateral lesions of the locus coeruleus (LC) markedly increased susceptibility to the cataleptogenic effects of neuroleptics. The apomorphine-induced stereotypy was enhanced in rats with lesioned LC whilst amphetamine stereotypy was only slightly increased. No changes in locomotor activity have been observed in LC-lesioned rats treated with apomorphine and amphetamine. This data indicates that lesions of the LC produce decreased activity of dopaminergic brain neurons as well as supersensitivity of dopaminergic receptors.

Locus coeruleus Dopamine Noradrenaline Neuroleptics Amphetamine Apomorphine

THE LOCUS coeruleus (LC) designated as cell group A-6 [10] contains noradrenergic cell bodies giving a diffuse ascending projection to cortical and probably to some subcortical brain areas [26,45]. Neuroanatomically the LC is classified as a part of the reticular formation [37]. It is possible that the noradrenaline (NA) containing neurons of the LC and their ascending fibres make up an important part of the ascending reticular activating system [26]. There is evidence that LC is important for induction and maintenance of paradoxical sleep and waking [7, 21, 22]. Recently, the possible involvement of the LC in motor activity regulating processes has been emphasized by some investigators [11,32]. Pyckok *et al.* [32] and Mc Donaldson *et al.* [11] reported that unilateral lesions of this area result in ipsilateral rotation which is then replaced by contralateral rotation. Moreover, in rats with unilateral LC lesion the rotation produced by apomorphine is contraversive to the lesioned site. This effect may be due to a decrease in ipsilateral nigrostriatal activity and consequently – due to supersensitivity of the dopaminergic receptors [11,46]. On the basis of this finding the lesion of the LC was postulated as causing interruption of a facilitative NA flow to the nigrostriatal pathway.

There are some experimental data indicating that NA may act as a transmitter in the region of the substantia nigra where the dopamine (DA) containing cell bodies giving ascending axons to the striatum are located. The measurable quantities of NA in this area as well as the NA nerve terminals have been found [12,14].

Some pharmacological evidences indicate indirectly that brain DA synthesis and utilization may be regulated through changes in NA neurotransmission [1,2].

In an attempt to obtain more informations about the possible role of the LC in activity of brain DA system we have studied the effects of drugs affecting DA pre- and postsynaptic mechanisms in rats with bilateral lesions of the LC. In the present study the cataleptogenic effects of some neuroleptics as well as amphetamine and apomorphine induced stereotypy and hypermotility were analysed.

METHOD

Animals and Operations

Male Wistar rats weighing 180–200 g were used. Animals were housed in macrolon cages 42 × 24 × 20 cm (3–4 per cage) at constant room temperature 21°C and 60% humidity. Lab. standard chow and water were available ad lib. Rats were anaesthetized using chloral hydrate (300 mg kg^{-1} IP) and their heads were positioned in a Stoelting stereotaxic apparatus with the incisors base angled 5° downward from the horizontal. The coordinates used for placement of the electrode tip in the LC were: P 1.6 mm (posterior to the interaural line), L 1.1 mm (lateral to the midline) and H 2.6 mm (above the interaural line). Electrolytic lesions were made using an 0.25 mm diameter stainless steel electrode as anode, varnished except for 0.5 mm at the tip. A stainless steel needle inserted into the tail was the cathode. Bilateral lesions of the LC were made using the current of 2.0 mA for 5–6 sec delivered through the anode. Sham-operated

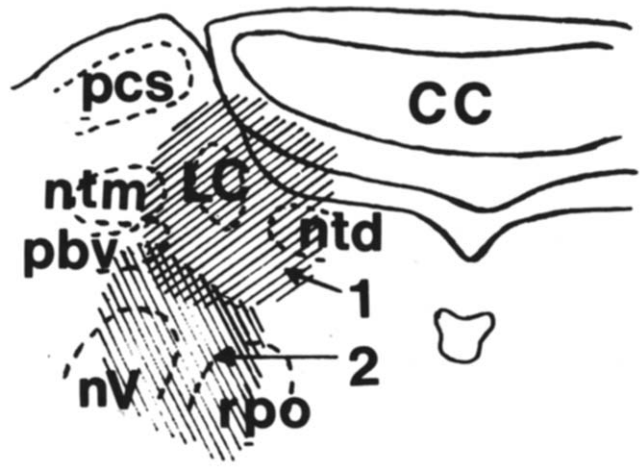

FIG. 1. Schematical representation of lesions involving the locus coeruleus (1) and lesions located outside this area (2). CC– cerebellar cortex, LC– locus coeruleus, ntd– nucleus tegmenti dorsolateralis, ntm– nucleus tractus mesencephalicus nervi trigemini, pcs– pedunculus cerebellaris superior, pbv– nucleus parabrachialis ventralis, nV– nucleus motorius nervi trigemini, rpo– nucleus reticularis pontis oralis.

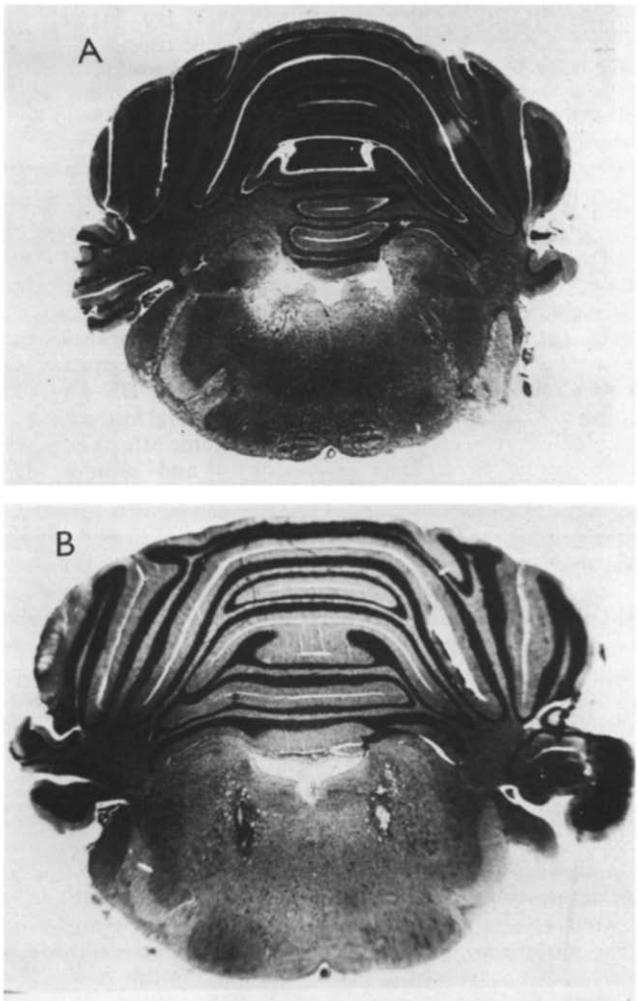

FIG. 2. Frontal sections showing a representative lesions involved LC (A) and lesions located outside this area (B).

animals were prepared by inserting the electrode 2 mm dorsal into this region yet not passing a current.

Histological and Biochemical Examinations

At the end of experiments animals were killed by decapitation, their brains were quickly removed and dissected by precollicular section (caudally to hypothalamus). The brainstems were checked histologically after fixation in 10% Formalin, the sections were stained with haematoxylin and eosin and by the Klüver-Barrera technique [23].

Biogenic amines levels were measured in separate group of animals. The extraction and fluorimetric determination of brain amines was carried out basically according to Haubrich and Denzer [17], the only difference being that 5-HT was determined according to Korf and Sebens [24].

Testing of Animals and Drugs

The effects of drugs were tested 8–9 days after lesions. The catalepsy and stereotypy were scored using methods described elsewhere [16,25]. The general motor activity was measured using the Activity Meter Type AM-1, IBP PAN licence, Poland.

The following drugs were used: apomorphine hydrochloride (Sandoz), d-amphetamine sulphate (Smith, Kline and French), chlorpromazine (POLFA), haloperiodol (Janssen) and spiroperidol (Janssen). All drugs except haloperidol and spiroperidol were dissolved in saline. The last drugs were dissolved by heating in 0.01 N tartaric acid and adjusted to pH 3.0. The doses and routes of administration are detailed with results of experiments. All experiments were performed always between 10:00 a.m. and 2:00 p.m.

Statistics

Statistical analysis was made using the Mann and Whitney two-tailed test (behavioral experiments) and Student's *t*-test, two-tailed (biochemical experiments).

RESULTS

Locations of Lesions

Histological examinations confirmed that the lesions of the LC were mainly restricted to this area medially to the superior cerebellar peduncle and mesencephalic root nucleus of trigeminal nerve, the edge parts of these structures were, however, also lesioned (Fig. 1). In some animals lesions were not accurately positioned and involved no LC but structures such as the nucleus motorius nervi trigemini, nucleus parabrachialis ventralis and partially, nucleus reticularis pontis oralis. If number of rats having such lesions was sufficient the separate group was formed and compared with sham-lesioned and LC-lesioned animals. The representative lesions involving the LC and lesions located outside of this area are shown in Fig. 2.

Biochemical Examinations

Rats with lesions located within the LC showed significantly decreased forebrain NA levels without changes in DA and 5-HT concentrations. Lesions not involving the LC failed to change brain amines concentrations (Table 1).

Testing of Drugs

As shown in Fig. 3 lesions involving the LC markedly

TABLE 1

BRAIN AMINE CONCENTRATIONS IN LESIONED AND CONTROL (SHAM-LESIONED) ANIMALS

Experimental group	n	Forebrain concentrations ng/g^{-1} (mean values ± SE)		
		Serotonin	Noradrenaline	Dopamine
Sham-lesioned	6	514.1 ± 56.6	339.4 ± 12.3	1076.1 ± 81.0
LC lesions	7	534.1 ± 48.6	175.4 ± 17.0*	983.3 ± 117.6
Lesions not involving LC	5	627 ± 43.8	293.3 ± 40.3	1138.8 ± 63.8

n = number of rats.
* = $p<0.01$ (Student $t$test).

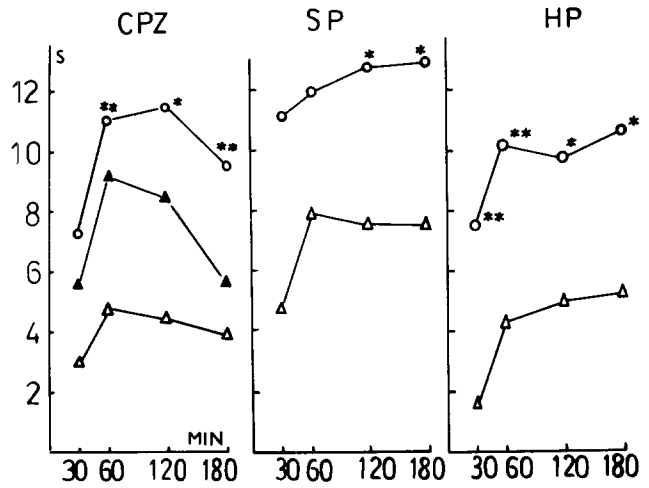

FIG. 3. Cataleptogenic effects of neuroleptics in rats. CPZ-chlorpromazine (5 mg kg^{-1} IP), SP-spiroperidol (1.5 mg kg^{-1} IP) and HP-haloperidol (0.5 mg kg^{-1} IP). ○——○ bilateral lesions involving LC, ▲——▲ lesions not involving LC, △——△ sham lesions. Values are means of 8–12 experiments ± SE. Ordinate-the intensity of catalepsy (scored), abscissa-time after injection in minutes. * = $p<0.05$, ** = $p<0.025$ (Mann and Whitney, two-tailed).

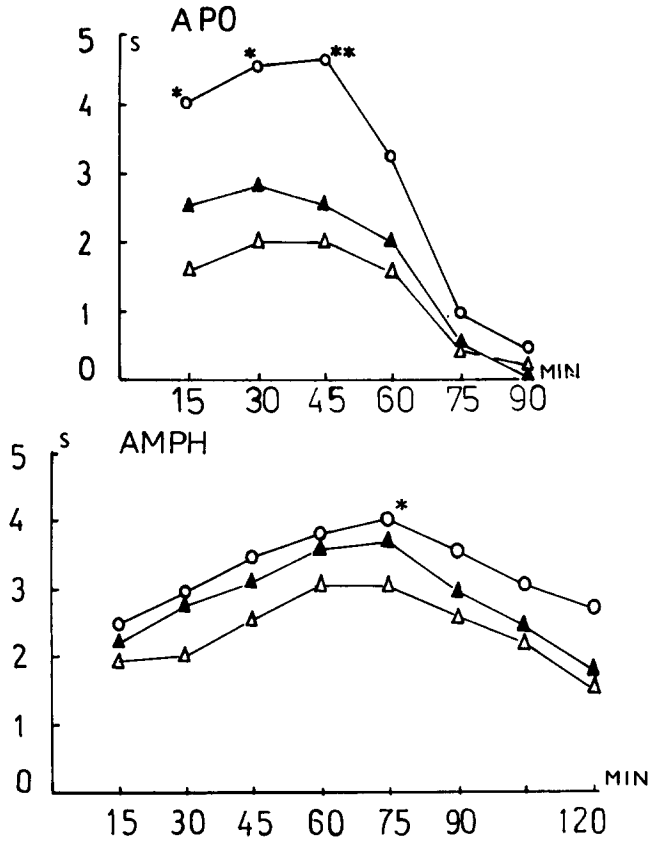

FIG. 4. The stereotype behavior induced by apomorphine (APO) (10 mg kg^{-1} IP) and amphetamine (AMPH) (10 mg kg^{-1} IP). Ordinate-the intensity of stereotypy (scored), abscissa-time after injection in mins. * = $p<0.025$, ** = $p<0.01$. Values are means of 6–8 experiments ± SE. ○——○ bilateral lesions of the LC, ▲——▲ lesions not involving LC, △——△ sham lesions.

potentiated the cataleptogenic effect of neuroleptics. This was true for chloropromazine (5 mg kg^{-1} IP) as well as for the butyrophenone derivatives-haloperidol (0.5 mg kg^{-1} IP) and spiroperidol (1.5 mg kg^{-1} IP). Lesions not involving the LC caused no changes in chlorpromazine-induced catalepsy.

Stereotypy was increased in rats with lesioned LC, this effect, however, was much stronger in apomorphine-treated rats than in amphetamine-treated animals (Fig. 4).

No changes in both – amphetamine-induced hypermotility and apomorphine-induced hypermotility were observed in rats after lesions of the LC.

DISCUSSION

The results of our experiments indicate that bilateral lesions of the NA system of the LC lead to increased cataleptogenic actions of neuroleptics as well as to enhancement in apomorphine and amphetamine stereotypy. The locomotor actions of amphetamine and apomorphine was unchanged.

Neuroleptics are thought to act primarily by blocking brain DA and NA receptors and by decreasing the release of DA from nerve terminals [3, 4, 36, 41, 48]. These drugs are also known to induce an increase in dopamine turnover as possible compensation to their receptor blocking activity [3, 4, 6]. It is generally accepted that butyrophenone derivatives such as haloperidol and spiroperidol have little effect on NA receptors when compared with chlorpromazine [3]. The behavioral effects of neuroleptics are markedly enhanced by the tyrosine hydroxylase inhibitor, α methyl-p-tyrosine (AMT) [5], the drug blocking the synthesis of both NA and DA [42]. The potentiating effects of AMT on the action of neuroleptic drugs is

probably due to its inhibition of neuroleptic-induced increase in catecholamine turnover, thereby reducing the amount of amine available to compete with the neuroleptic drugs for receptor sites [5]. On the basis of this finding we may suppose that enhancement of neuroleptic-induced catalepsy after bilateral lesions of the LC is due to reducing the activity of the DA brain neurons. Since catalepsy induced by neuroleptic drugs is mainly due to their action upon DA nigro-striatal system it may be possible that lesion of the LC leads to a decreased impulse flow in the DA nigro-striatal neurons.

The results obtained with apomorphine and amphetamine support this hypothesis. It is possible that increased apomorphine and amphetamine stereotypy in rats with lesioned LC is because of increased sensitivity of DA nigro-striatal receptors. A number of workers have suggested that stereotypy is mediated through DA neurons whilst both DA and NA neurons seem to be related to locomotor behavior [15, 29, 33, 34, 43, 44]. Amphetamine acts through endogenous catecholamines whilst apomorphine acts mainly directly upon postsynaptic DA receptors [13,28]. It is worthwhile to point out that increased apomorphine effects have been observed in rats with lesioned DA nigrostriatal system [31,46]. Similar effect was observed after intraventricular administration of 6-hydroxydopamine [39] the compound which selectively destroys catecholamine-containing nerve-terminals [20,38]. Schoenfeld and Uretsky [39] have observed the enhanced effects of l-DOPA (3,4-dihydroxyphenylalanine) in rats treated with 6-hydroxydopamine. All effects described above may be explained as a sign of DA receptors hypersensitivity [40,47]. The apomorphine-induced stereotypy was in our study potentiated to a much greater extent than amphetamine-induced stereotypy. This phenomenon may be explained on the basis of well known postsynaptic site of action of apomorphine in contrast with the presynaptic site of action of amphetamine [6]. The slight enhancement in the amphetamine stereotypy indicates that the amount of DA released by this drug was sufficient to produce the behavioral effect stronger than normally.

It is noteworthy that locomotor effects of both amphetamine and apomorphine were similar in sham-lesioned and in LC-lesioned rats. This finding indicates that catecholamine neurons controlling this behavioral pattern remained rather unchanged. It is known that pretreatment with dopamine-hydroxylase inhibitors such as FLA-63 (bis-4-methyl-l-homopiperazinyl-disulphide) and U-14 624, being accompanied by a decrease in NA brain content affected only partially or not at all amphetamine-induced locomotor activity [6, 8, 15, 18]. On the other hand pretreatment of mice and rats with AMT blocks the increased locomotor activity and stereotyped behavior produced by amphetamine [15,44]. In contrast to results obtained with FLA-63 and U-14, 624, diethyldithiocarbamate, which acts at least in part to inhibit DA hydroxylase did block the increase in locomotor activity induced by amphetamine [35].

According to our findings it may be concluded that bilateral lesions of the LC lead to decreased nerve impulse flow in DA neurons and to increased sensitivity of DA receptors. Therefore, it may be probable that LC normally facilitates the DA neurons in the brain. There is open question whether other than nigro-striatal DA neurons may be functionally connected with LC. Recent studies provide evidence that so-called mesolimbic dopaminergic system plays an important role in the locomotor activity in rats [9, 19, 30].

It is also worthwhile noting that apomorphine-induced aggression in rats is markedly potentiated after bilateral lesion of the LC (Kostowski and Jerlicz, unpublished). Since DA is believed to be involved in the mechanisms of aggression we may suppose that not only motor but also emotive effects of DA systems are physiologically facilitated by NA neurons of the LC. Such an interaction between the DA and NA neuronal systems might be of importance for mechanisms of action of psychotropic drugs.

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