Serotoninergic modulation of the dopamine response from the nucleus accumbens

BRENDA COSTALL, ROBERT J. NAYLOR†, C. DAVID MARSDEN*, CHRISTOPHER J. PYCOCK*, *Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire, U.K. and* Department of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London, U.K.*

The depletion of brain 5-hydroxytryptamine (5-HT) by electrolytic lesions of the midbrain raphé nuclei, or by synthesis inhibition, results in marked hyperactivity in the rat (Kostowski, Giacolone & others, 1968; Jacobs, Trimbach & others, 1975). This would suggest that 5-HT may normally participate in motor control by exerting a powerful inhibitory effect. The nature of the system or systems subjected to such a control is not known but catecholaminergic mechanisms have been shown to be essentially involved in the regulation of motor activities and some workers have suggested a possible relation between these controlling mechanisms and 5-HT function (Neill, Grant & Grossman, 1972; Costall & Naylor, 1974). A site for such an interaction is suggested by recent studies showing that a marked hyperactivity results from an increased dopaminergic activity within the nucleus accumbens (Pijnenburg, Honig & van Rossum, 1975; Costall & Naylor, 1975), and this nucleus is known to be innervated by serotoninergic as well as by dopaminergic neurons (Fuxe, 1965; Conrad, Leonard & Pfaff, 1974). Therefore, we investigated the possible role of the nucleus accumbens in the hypothesized dopaminergic/serotoninergic control of motor function.

Bilateral stainless-steel guide cannulae (0·65 mm diameter) were chronically implanted, using stereotaxic techniques, to allow the direct injection into the nucleus accumbens of male, Sprague-Dawley rats (250–300 g). Chloral hydrate (300 mg kg^{-1}, i.p.) was used as anaesthetic. 0·3 mm diameter stainless-steel stylets terminated 0·5 mm below the guide tips and kept the guides patent. Animals were subjected to intracerebral injections 10–14 days after surgery when they were manually restrained as the stylets were replaced by stainless-steel injection units which terminated 2·5 mm below the guides at the centre of the nucleus accumbens (Ant. 9·0, Vert. 0, Lat. ±1·6) (De Groot, 1959). Injection units were coupled to Agla micrometer syringes and 2 μl drug or solvent solution was delivered bilaterally over 5 s with a further 55 s allowed for deposition of drug. Rats were used on two occasions with an intervening 7–10 day recovery period, and were then killed for histological and biochemical examination.

In some experiments, lesions were made in the medial raphé nucleus on the same occasion as implantation of the guides. These lesions were induced electrolytically using a stainless-steel electrode, 0·64 mm diameter, which was insulated excepting at the tip. The electrode was set at an angle of 65° posterior, lowered to Ant. 0·3, Vert. −2·6, Lat. 0 (Sham Vert. −1·6) (König & Klippel, 1963) and the lesion induced by passing a current of 1·0 mA for 10 s, the stereotaxic frame serving as the inert electrode. Sham-operated animals were similarly treated but no current was passed.

All activity experiments were carried out between 08·00 and 18·00 h in a sound-proofed, diffusely illuminated room at 21 ± 1°. Activity boxes, 30 cm × 20 cm and 15 cm high, were fitted with photocells. Activity was measured by counting the number of interruptions of the light beam occurring during each 5 min period. Animals were placed in the boxes immediately following the injection of drug or solvent into the nucleus accumbens and activity counts recorded for a maximum of 5 h. All animals were given nialamide (100 mg kg^{-1}, i.p.) 2 h before the intracerebral injections (see Costall, Naylor & Pinder, 1974, for the rationale of the nialamide pretreatment).

For biochemical determinations rats were killed by cervical dislocation under light chloroform anaesthesia, the brains rapidly removed and dissected over ice. The midbrain was separated from the forebrain for histological examination. In all experiments, the various forebrain regions from each side were pooled and assayed for biogenic amine content. Cortical slices were taken from the fronto-parietal cortex and were used for the determination of noradrenaline and 5-HT. The striata were dissected out for dopamine and 5-HT assay, and the area of the nucleus accumbens and tuberculum olfactorium (König & Klippel, 1963) was taken for dopamine and 5-HT determination.

The separate brain regions were immediately weighed and homogenized in cold acidified butanol for the extraction of biogenic amines. 5-HT was determined fluorometrically following condensation with *o*-phthaldehyde (Maickel, Cox & others, 1968). Noradrenaline was assayed fluorometrically (Chang, 1964) as was dopamine following purification by alumina and subsequent condensation with ethylene diamine (Laverty & Sharman, 1965).

Dopamine hydrochloride (Koch-Light) was prepared for intracerebral injection in nitrogen-bubbled distilled water neutralized with sodium bicarbonate, and 5-HT bimaleinate (Koch-Light) was dissolved in distilled water: both were prepared immediately before use. Nialamide (Sigma) for intraperitoneal injection was dissolved in a minimum quantity of hydrochloric acid and made up to volume with distilled water. All doses were calculated as the base.

† Correspondence.

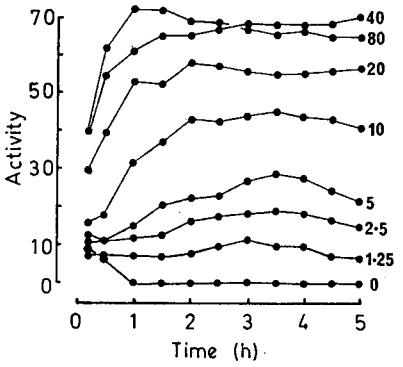

FIG. 1. Dose-dependency of the hyperactivity induced by dopamine injected directly into the nucleus accumbens of normal rats 2 h after the intraperitoneal administration of 100 mg kg^{-1} nialamide. Doses are expressed as μg per 2 μl administered bilaterally. Each value is the mean of determinations from 6 to 8 rats. Standard errors are in the range 9 to 17% of the means. y-axis-Activity (counts/5 min).

The bilateral administration of dopamine into the nucleus accumbens of rats pretreated with nialamide caused a dose-dependent hyperactivity which was apparent at 1·25 μg and maximal at 40 μg (Fig. 1). The intensity of this hyperactivity was increased in rats with lesions of the medial raphé nucleus, when the threshold dose was shown to be 0·625 μg and a maximum response was recorded at 5 μg (Fig. 2). However, animals with lesions of the medial raphé nucleus exhibited higher basal activity levels (see also Costall, Fortune & others, 1975) and, although a comparison of the maximal effects at 1·25, 2·5 and 5 μg dopamine between lesioned and sham-operated rats showed a highly significant potentiation of the dopamine response ($P<0·001$ at each dose level), such a difference could only be determined for the threshold doses if calculations were made not with reference to absolute values but in terms of increases over basal activity.

In addition to changes in the intensity of response to dopamine injected into the nucleus accumbens, it was noticeable that the time course of the dopamine response was also altered by the medial raphé nucleus lesions: in normal rats there is usually a delay of some 90–120 min to attainment of the maximum response, but the lesioned rats exhibited increased activity within 5–10 min of the dopamine injection and were responding maximally after 30–60 min. However, the maximal response was maintained for a significantly longer time period in non-lesioned or sham-operated rats ($P<0·001$ at all doses tested).

Doses of 5-HT ranging from 0·1–100 μg injected bilaterally into the nucleus accumbens of normal rats, either with or without a nialamide pretreatment, failed to elicit an activity response. In contrast, the animals appeared depressed and the usual exploratory behaviour was generally absent. Further, when 5-HT, 6·25 μg or more, was injected bilaterally into the

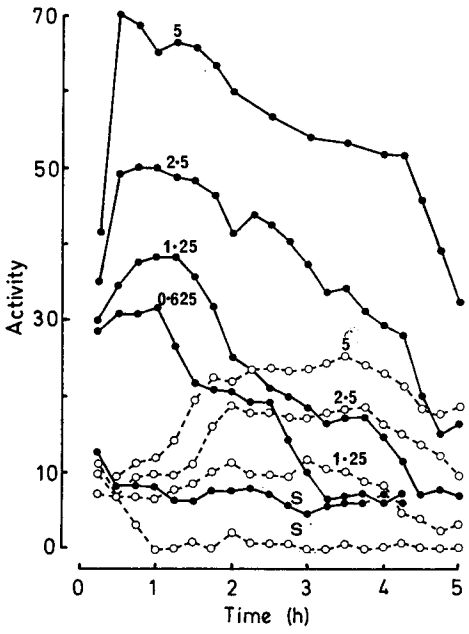

FIG. 2. Increase in the hyperactivity response to bilateral injections of dopamine into the nucleus accumbens following lesion of the medial raphé nucleus. ●——● hyperactivity response to dopamine in lesioned animals, ○ - - - ○ response of sham-operated animals. Doses of dopamine are expressed as μg per 2 μl administered bilaterally. S—response of animals receiving bilateral injections into the nucleus accumbens of 2 μl solvent. The effects of 0·625 μg dopamine in sham-operated rats were indistinguishable from solvent effects in the same group of animals. All rats were given nialamide (100 mg kg^{-1}, i.p.), 2 h before the intracerebral injections. Experiments were carried out between the 10th and 24th postoperative days, lesions and cannulae implantations being performed on the same occasion. 6 to 8 rats were used at each dose level and standard errors on the means are within the range 11 to 19% of the mean. y-axis-Activity (counts/5 min).

nucleus accumbens of rats exhibiting hyperactivity after a prior injection of dopamine, a dose-dependent reduction in the hyperactivity response was recorded and the effects of 50 μg dopamine were completely inhibited by 25 μg 5-HT (Fig. 3) (antagonistic action of 5-HT significant to $P<0·001$ at 6·25 μg and above). The reduction in activity was apparent within 3–10 min of the 5-HT injections and persisted throughout the normal response to dopamine (6–7+ h). This ability of 5-HT to antagonize the hyperactivity response to dopamine was significantly reduced in animals with lesions of the medial raphé nucleus ($P<0·01$–$P<0·001$) (Fig. 3).

The results of the biochemical examinations of the lesioned brains are in Table 1. The medial raphé nucleus lesions produced a significant and approximately equal reduction of 5-HT concentrations in the

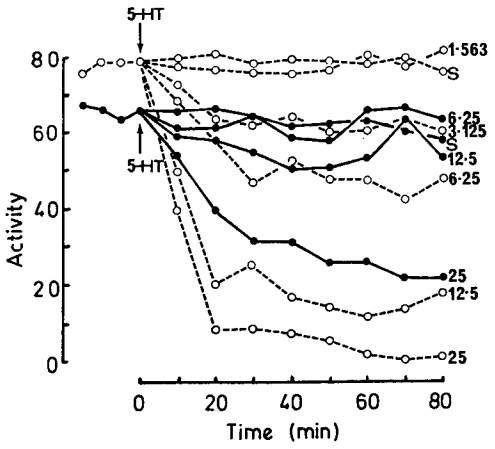

FIG. 3. The ability of 5-HT, injected bilaterally into the nucleus accumbens, to reduce the hyperactivity response to previous bilateral injections of dopamine into the same nucleus, and the reduction in this effect of 5-HT following lesion of the medial raphé nucleus. ○----○ responses of sham-operated animals—5-HT was administered bilaterally into the nucleus accumbens 3 h after similar bilateral injections of 50 μg dopamine, at a time when the hyperactivity response to dopamine was established at maximum. ●——● response of animals with lesions of the medial raphé nucleus—5-HT was administered bilaterally into the nucleus accumbens 1 h after a similar bilateral injection of 5 μg dopamine, at a time when the hyperactivity response to dopamine was maximum. Doses of 5-HT are expressed as μg per 2 μl. S—control animals receiving solvent instead of 5-HT. All animals were given nialamide (100 mg kg^{-1}, i.p.) 2 h before the injections of dopamine. Experiments were carried out between the 10th and 24th postoperative days, lesions and cannulae implantations being performed on the same occasion. 6 to 8 rats were used at each dose level and standard errors on the means are within the range 9 to 15% of the mean. y-axis-Activity (counts/5 min).

cerebral cortex—59%, striatum—47% and limbic forebrain—49%. In contrast the lesions had no significant effect ($P>0{\cdot}01$) on either dopamine concentrations in the striatum and limbic forebrain, or on noradrenaline concentrations in cerebral cortex.

Histological examination of the lesion site showed extensive damage in the region of the medial raphé nucleus which extended to the pedunculus cerebellaris superior and tractus tectospinalis, although the mesencephalic reticular formation was only rarely damaged (Fig. 4). Those brains examined to establish the location of injection sites in the nucleus accumbens all showed a correct location for injection into the area of the nucleus accumbens (Fig. 4).

An important role for the nucleus accumbens in the initiation or modulation of motor function is indicated by the marked hyperactivity induced by dopamine, noradrenaline and other phenethylamines after injection into this area of the brain (Pijnenberg & van Rossum, 1973; Costall, Naylor & Pinder, 1976). The results of studies on the effects of similar injections into closely surrounding areas indicate that these marked effects are mediated from the nucleus accumbens. For example, dopamine applied to the anatomically adjoining tuberculum olfactorium or caudate-putamen can induce a hyperactivity but of much lower intensity (Costall & Naylor, 1975; Jackson, Andén & Dahlström, 1975). The action of dopamine and related agents in the nucleus accumbens is specifically antagonized by neuroleptic agents (Pijnenburg, Honig & van Rossum, 1975; Costall & Naylor, 1976) and this suggests that the mechanisms involved are dopaminergic. It would appear reasonable to assume that, in normal animals, this powerful dopaminergic facilitatory effect on locomotor activity should be subject to some inhibitory influence, and the present studies indicate that the serotoninergic innervation to the nucleus accumbens may provide the requisite inhibitory function.

The ascending telencephalic serotoninergic systems have long been considered to exert a general inhibitory action on motor function (Brodie & Shore, 1957), and the neurons ascending from the medial raphé nucleus are thought to play a particularly important role (Srebro & Lorens, 1975; Costall & others, 1975).

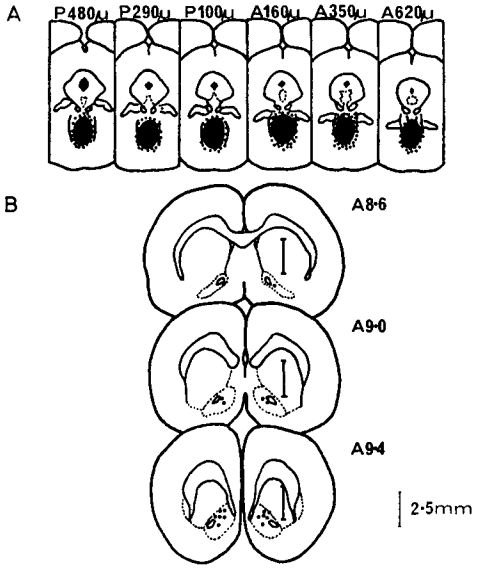

FIG. 4. Diagrammatic representation of the location of A, electrolytic lesions in the area of the medial raphé nucleus (black shading represents tissue damage observed in all sections examined and stippled shading represents areas where damage was occasionally observed), coordinates according to the atlas of König & Klippel (1963), and B, injection sites (●) in the nucleus accumbens, coordinates according to De Groot (1959). The diagrams were constructed from the histological data obtained from the brains of 10 rats.

Table 1. *Effects of lesions of the medial raphé nucleus (MRN) on the 5-HT, dopamine and noradrenaline content of the cerebral cortex, striatum and limbic regions (nucleus accumbens septi and tuberculum olfactorium).*

	5-HT (ng g^{-1} wet wt ± 1 s.e.m.)			Dopamine (μg g^{-1} wet wt ± 1 s.e.m.)		Noradrenaline (ng g^{-1} wet wt ± 1 s.e.m.)
Lesion	Cortex	Striatum	Limbic	Striatum	Limbic	Cortex
None	390 ±27	685 ±43	644 ±50	2·92 ±0·21	1·29 ±0·16	329 ±20
MRN	160 ±21	360 ±54	327 ±43	3·22 ±0·17	1·33 ±0·08	297 ±21
%	41***	53***	51***	110	103	90

The mean values are given for 7 or 8 determinations and changes caused by the lesion are expressed as a percentage of control values. The significance of these changes were determined using the Students' *t*-test, $*P<0.01$, $**P<0.005$, $***P<0.001$.

Although the serotoninergic system arising from this raphé nucleus supplies many forebrain structures, the present studies considered the innervation to the nucleus accumbens. In this region, 5-HT was shown to play a critical role in the modulation of locomotor activity. Thus, 5-HT injections into the nucleus accumbens were shown to reduce the hyperactivity response to prior injections of dopamine in a dose-dependent manner, and, at larger doses, to completely abolish the dopamine effect. Further, when forebrain concentrations of 5-HT were specifically reduced following lesions of the medial raphé nucleus the hyperactivity responses to dopamine injections were markedly increased.

In these latter experiments it was noted that whilst the medial raphé nucleus lesions resulted in an increased facilitatory effect of dopamine on activity, they also reduced the inhibitory effect of 5-HT. There are arguments in the literature in favour both of a decreased receptor sensitivity and the development of receptor supersensitivity following a lesion-induced reduction in nerve impulse flow, there may be changes in receptor specificity, even compensatory changes in other receptor systems within the same or associated areas. Although our biochemical estimations failed to show a reduction in telencephalic dopamine concentrations following the raphé lesions, this does not preclude changes in dopamine turnover. Thus, any comment on the mechanisms of the decrease in the 5-HT effect would be highly speculative but if changes in receptor sensitivity do occur as a result of the lesion, they may involve sites sensitive to 5-HT and/or dopamine.

Whatever the complexities of the receptor mechanisms involved, the present studies indicate that, within the nucleus accumbens, the facilitatory effect of dopamine on locomotion may be modulated by an inhibitory influence from serotoninergic mechanisms. This observation has clear implications both for our understanding of the aetiology of motor disorders and for their treatment.

This work was supported by the Medical Research Council and the Parkinson's Disease Society. Miss M. Y. Green and Mr. C. Brewer gave excellent technical assistance.

January 12, 1976

REFERENCES

BRODIE, B. & SHORE, P. (1957). *Ann. N.Y. Acad. Sci.*, **66**, 631–642.
CHANG, C. C. (1964). *Int. J. Neuropharmac.*, **3**, 643–649.
CONRAD, L. C. A., LEONARD, C. M. & PFAFF, D. W. (1974). *J. Comp. Neur.*, **156**, 179–206.
COSTALL, B. & NAYLOR, R. J. (1974). *Eur. J. Pharmac.*, **29**, 206–222.
COSTALL, B. & NAYLOR, R. J. (1975). *Ibid.*, **32**, 87–92.
COSTALL, B. & NAYLOR, R. J. (1976). *Ibid.*, in the press.
COSTALL, B., NAYLOR, R. J. & PINDER, R. M. (1974). *J. Pharm. Pharmac.*, **26**, 753–762.
COSTALL, B., FORTUNE, D. H., NAYLOR, R. J., MARSDEN, C. D. & PYCOCK, C. (1975). *Neuropharmac.*, **14**, 859–868.
COSTALL, B., NAYLOR, R. J. & PINDER, R. M. (1976). *Psychopharmacologia (Berl.)*, in the press.
DE GROOT, J. (1959). *Verh. K. Ned. Akad. Wet.*, **52**, 14–39.
FUXE, K. (1965). *Acta physiol. scand., Suppl.*, **247**, 37–85.
JACKSON, D. M., ANDÉN, N.-E. & DAHLSTRÖM, A. (1975). *Psychopharmacologia, (Berl.)*, **45**, 139–149.
JACOBS, B. L., TRIMBACH, C., EUBANKS, E. E. & TRULSON, M. (1975). *Brain Res.*, **94**, 253–261.
KÖNIG, J. F. R. & KLIPPEL, R. A. (1963). *The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins Co.
KOSTOWSKI, W., GIACOLONE, E., GARATTINI, S. & VALZELLI, L. (1968). *Eur. J. Pharmac.*, **4**, 371–376.
LAVERTY, R. & SHARMAN, D. F. (1965). *Br. J. Pharmac.*, **24**, 538–548.
MAICKEL, R. P., COX, R. N., SAILLANT, J. & MILLER, F. P. (1968). *Int. J. Neuropharmac.*, **7**, 275–281.
NEILL, D. B., GRANT, L. D. & GROSSMAN, S. P. (1972). *Physiol. Behav.*, **9**, 655–567.
PIJNENBURG, A. J. J., HONIG, W. M. & VAN ROSSUM, J. M. (1975). *Psychopharmacologia (Berl.)*, **41**, 175–180.
PIJNENBURG, A. J. J. & VAN ROSSUM, J. M. (1973). *J. Pharm. Pharmac.*, **25**, 1003–1005.
SREBRO, B. & LORENS, S. A. (1975). *Brain Res.*, **89**, 303–325.