concentrations are correlated much better with the capacity of these drugs to release 5-HT from platelets *in vitro* than with their *in vitro* inhibitory activity on platelet 5-HT uptake.

The invaluable assistance of Miss Maria Carla Roncaglioni is gratefully acknowledged.

Istituto di Ricerche Farmacologiche
'Mario Negri',
Via Eritrea, 62
20157 Milan, Italy

W. BUCZKO*
G. DE GAETANO**
S. GARATTINI

December 13, 1974

* Visiting Scientist from the Department of Pharmacology, Medical School, Bialystok, Poland
** To whom reprint requests should be addressed.

REFERENCES

BECKETT, A. H., SHENOY, E. V. B. & BROOKES, L. G. (1972). *J. Pharm. Pharmac.*, **24**, 281–288.
BUCZKO, W., DE GAETANO, G. & GARATTINI, S. (1974). *Ibid.*, **26**, 814–815.
BUCZKO, W., DE GAETANO, G. & GARATTINI, S. (1975). *Br. J. Pharmac.*, in the press.
DA PRADA, M., BARTHOLINI, G. & PLETSCHER, A. (1965). *Biochem. Pharmac.*, **14**, 1721–1726.
DUHAUFT, J. & VERDAVAINNE, C. (1967). *Archs int. Pharmacodyn. Thér.*, **170**, 276–286.
FULLER, R. W., HINES, C. W. & MILLS, J. (1965). *Biochem. Pharmac.*, **14**, 483–488.
GARATTINI, S., BIZZI, A., DE GAETANO, G., JORI, A. & SAMANIN, R. (1974). Paper presented at the 1st. International Congress on Obesity, London, October, 9–11.
GROPPETTI, A., MISHER, A., NAIMZADA, M., REVUELTA, A. & COSTA, E. (1972). *J. Pharmac. exp. Ther.*, **182**, 464–473.
PLETSCHER, A., BARTHOLINI, G., BRUDERER, H., BURKARD, W. P. & GEY, K. F. (1964). *Ibid.*, **145**, 344–350.
RICHTER, A. & SMITH, S. E. (1974). *J. Pharm. Pharmac.*, **26**, 763–770.
SANDERS-BUSH, E. & SULSER, F. (1970a). In *Amphetamines and Related Compounds*, pp. 349–355. Editors: Costa, E. & Garattini, S., New York: Raven Press.
SANDERS-BUSH, E. & SULSER, F. (1970b). *J. Pharmac. exp. Ther.*, **175**, 419–426.

The role of the raphé and extrapyramidal nuclei in the stereotyped and circling responses to quipazine

The induction of stereotyped behaviour patterns in laboratory animals is widely used as an index of dopaminergic stimulation and, therefore, as an indicator of antiparkinson potential (Costall, Naylor & Wright, 1972; Fog, 1972; Costall, Naylor & Pinder, 1974). However, the ability of dopaminergic agonists to cause stereotypy has also recently been associated with serotoninergic (5-HT) mechanisms (Costall & Naylor, 1974a) and this raises questions as to the possible relation between dopamine and 5-HT in parkinsonism. Reports that quipazine causes stereotyped behaviour patterns but acts mainly upon 5-HT mechanisms (Rodriguez & Pardo, 1971; Medon, Leeling & Phillips, 1973; Grabowska, Antkiewicz & Michaluk, 1974) are, therefore, of great interest and stimulated the present studies to investigate its action using brain lesion and intrastriatal injection techniques.

Male Sprague-Dawley rats, 250—300 g, were used. In these animals quipazine (2-(1-piperazinyl) quinoline maleate) (Miles Laboratories) did not induce behavioural effects analogous to the stereotypy induced by known dopaminergic agonists, for example, apomorphine and (+)-amphetamine. The intensity of stereotyped behaviour induced by the latter agents generally increases with increasing dosage such that the weak intensity components of sniffing and repetitive limb movements are apparent at lower doses, and the intense components of gnawing, biting and licking at higher doses

(Costall & Naylor, 1973a, 1974b). However, at all doses of quipazine (5 to 40 mg kg^{-1}, s.c.) the nature of the behaviour was shown to be identical (sniffing, repetitive front limb movements, munching) and periodic in appearance. Only the periodicity of the behaviour changed with dose. Therefore, stereotypy was assessed simply as present or absent and 10 mg kg^{-1}, s.c., quipazine was used in all studies because it induced the most marked and consistent response. After this dose, periodic munching (also hyperactivity) developed within 1 to 2 min and within the following few minutes animals exhibited periods of sniffing and repetitive front limb movements, also a mild tremor was occasionally observed. After 10 to 15 min the periodicity of the behaviour increased and the hyperactivity ceased, animals frequently lying flat at the bottom of the cage. Periodic munching and occasional repetitive movements of the front limbs were apparent for at least a further 2 h. However, only the behaviour occurring during the first 10 to 15 min was of sufficient intensity for assessment in the drug interaction and brain lesion studies.

The stereotypic response to quipazine was determined following a 30 min pretreatment with haloperidol (Janssen) and cyproheptadine hydrochloride (Merck, Sharpe & Dohme) and in animals with bilateral lesions of the extrapyramidal nuclei (caudate-putamen, globus pallidus, substantia nigra), dopaminergic pathways supplying them (in lateral hypothalamus) or in the midbrain raphé nuclei (medial and/or dorsal raphé nucleus).

Lesions were induced using the method of electrolytic coagulation and the techniques and lesion coordinates previously described. The effects of the lesions were determined on the 6th to 24th postoperative days. On completion of the experiments the lesion locations were found to be indistinguishable from those previously described (Costall & Naylor, 1973a, b, c; 1974a, c). For stereotypy studies lesions were induced bilaterally (extrapyramidal areas) or centrally (raphé nuclei). For circling studies lesions were induced unilaterally in the substantia nigra and asymmetrically in the medial raphé nucleus.

Circling was measured as rev min^{-1} made by an animal in the 1 min immediately after it had been placed in a circular cage 40 cm diameter.

Intrastriatal injections were as described by Costall & Naylor (1974d). Briefly, stainless-steel guide cannulae were implanted 7 to 10 days before injection and kept patent by stainless-steel stylets. Injection cannulae were constructed to extend 1·5 mm below the tip of the guide and terminate at the centre of the caudate-putamen. Quipazine was delivered in a volume of 1 or 2 μl over 5 s with the injection cannula remaining in position for a further 55 s. The experimental techniques for demonstrating dopamine-like activity in the striatum after intrastriatal injection have been established. Briefly, contralateral turning or asymmetries are initiated by unilateral striatal dopamine stimulation in the presence of haloperidol pretreatment (2 mg kg^{-1}, i.p. 30 min) (Costall & Naylor, 1974d) and a stereotyped/hyperactive behaviour results after bilateral stimulation of striatal dopamine mechanisms in the presence of nialamide pretreatment (100 mg kg^{-1}, i.p. 2 h) (Costall & others, 1974). Locations of injection cannulae were indistinguishable from those previously reported (Costall & Naylor, 1974d).

All agents were administered peripherally by the intraperitoneal route excepting quipazine which was more effective when given subcutaneously. Quipazine was dissolved in distilled water, haloperidol in 1% lactic acid, nialamide (Sigma) in a minimum quantity of HCl made up to volume with distilled water, and cyproheptadine was prepared as an aqueous suspension in 2% carboxymethylcellulose.

The stereotypic activity of quipazine was not modified by the usual antistereotypic doses of haloperidol (Costall & Naylor, 1974e). A reduction in effect was observed only when quipazine was administered to animals exhibiting catalepsy under the

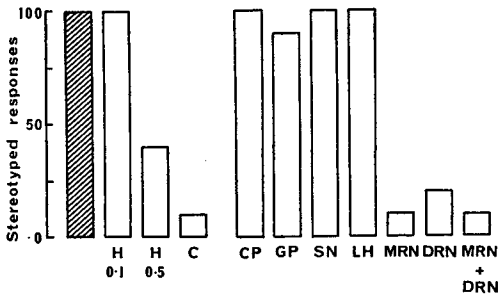

FIG. 1. Modification of the stereotyped responses induced by quipazine, 10 mg kg^{-1}, s.c., following pretreatment with haloperidol (H), 0·1 or 0·5 mg kg^{-1} i.p., or cyproheptadine (C), 10 mg kg^{-1} i.p., and following bilateral lesions of the caudate-putamen (CP), globus pallidus (GP), substantia nigra (SN) and dopamine pathways in the lateral hypothalamus (LH) or centrally placed lesions of the medial raphé nucleus (MRN) and/or dorsal raphé nucleus (DRN). Stereotypy was determined as the number of animals responding and is expressed as a % of the control values (hatched column). No difference could be determined between the responses of normal and sham-operated animals. At least 10 animals were used in each experiment.

influence of haloperidol (0·5 mg kg^{-1}, i.p.). The intensity of the catalespy was reduced by quipazine. In contrast, pretreatment with cyproheptadine (10 mg kg^{-1}, i.p.) markedly reduced the stereotypic action of quipazine (Fig. 1).

Lesions placed in the caudate-putamen, globus pallidus, substantia nigra or lateral hypothalamus did not modify the quipazine effect although this was markedly reduced by lesions of the medial and/or dorsal raphé nucleus (Fig. 1).

Animals with unilateral lesions of the substantia nigra did not exhibit a consistent circling behaviour after quipazine administration, 5 to 20 mg kg^{-1}, s.c., although a dose-dependent contralateral circling was observed following quipazine administration to animals with asymmetric lesions of the medial raphé nucleus (12 to 14 rev min^{-1} at 10 and 20 mg kg^{-1} s.c. quipazine).

The unilateral intrastriatal administration of quipazine, 12·5 to 50 μg, caused marked contralateral asymmetries in the presence of haloperidol, 2 mg kg^{-1}, i.p. At 12·5 μg, rats peridocially held the head to the side contralateral to the injection and tended to move in the same direction when disturbed although capable of movements straight forward. At 25 and 50 μg the asymmetries were more intense: the head and body were held continuously to one side and there was marked resistance to the manual turning of the body in the opposite direction. At all doses the asymmetries were apparent immediately following the 2 to 5 min period of injection artifact (see Costall & others, 1974) and lasted for at least 5 h.

The action of all dopaminergic agonists is dependent, to some extent, on functioning of the extrapyramidal nuclei or the neuronal pathways supplying them. For example, the stereotypic actions of apomorphine are markedly reduced or abolished by lesions to the dopaminergic pathways in the lateral hypothalamus, or by lesions of the globus pallidus or substantia nigra (Costall & Naylor, 1973a). Lesions of the globus pallidus also abolish the effects of (+)-amphetamine, (−)-amphetamine, ET495 1-(2″-pyrimidyl)-4-piperonyl-piperazine and methylphenidate (Costall & Naylor, 1973d; 1974b, f). In contrast, the effects of quipazine were not markedly modified by lesions of the caudate-putamen, globus pallidus, substantia nigra or lateral hypothalamus. Further, all dopaminergic agonists cause a circling behaviour in rats with unilateral lesion of the substantia nigra (Costall & Naylor, 1974g). Quipazine did not. Also, doses of the dopamine receptor blocking agent haloperidol which antagonize the actions of other dopaminergic agonists (Costall & Naylor, 1974e) were inactive against quipazine: the effects of quipazine were only antagonized by doses of haloperidol

which caused catalepsy and, therefore, interfered with the normal ability of the animal to initiate movement.

Thus, the integrity of dopaminergic mechanisms would not appear essential for the actions of quipazine. Nevertheless, this agent induces behavioural effects which, although of weak intensity, resemble those of known dopaminergic agonists. These effects were markedly reduced or abolished by lesions of the mid-brain raphé nuclei. Also, quipazine caused a circling behaviour in animals with asymmetric lesions of the medial raphé nucleus which, although of weaker intensity, compared with that induced by apomorphine, amphetamine and other dopaminergic agonists (Costall & Naylor, 1974a). Quipazine would appear dependent for its effect on functioning of the raphé nuclei and, thus, 5-HT systems.

Both brain lesion and intracerebral injection studies would indicate that, in contrast to the dopaminergic agonists, the striatum is not an important site for stereotypy induction by quipazine, although this area does contain large concentrations of both dopamine and 5-HT. Possibly the 5-HT innervation to the mesolimbic areas, also shown to be important for stereotypy induction (Costall & Naylor, 1973a; 1974b), may play a major role in the quipazine effect. In contrast, the striatum was indicated as a site for initiation of contralateral asymmetries. However, interpretational difficulties arise since contralateral asymmetries induced under the conditions of the present experiments have been shown to be specific for dopamine and dopamine-like agents (Costall & Naylor, 1974d) although the ability of haloperidol to enhance the asymmetries is abolished by raphé lesions (Costall & Naylor, 1974a). It is clear that the functions of dopamine and 5-HT are closely related and the present results indicate that stereotyped behaviour and contralateral asymmetries or circling may not simply be a measure of dopamine agonist properties but may also be representative of drug action on 5-HT mechanisms.

This work was supported by the Medical Research Council. Quipazine was kindly provided by Miles Laboratories and haloperidol by Janssen Pharmaceutica.

Postgraduate School of Studies in Pharmacology,
University of Bradford,
Bradford, West Yorkshire, U.K.

B. COSTALL
R. J. NAYLOR

November 15, 1974

REFERENCES

COSTALL, B. & NAYLOR, R. J. (1973a). *Eur. J. Pharmac.*, **24**, 8–24.
COSTALL, B. & NAYLOR, R. J. (1973b). *Br. J. Pharmac.*, **49**, 29–36.
COSTALL, B. & NAYLOR, R. J. (1973c). *Arzneimittel-Forsch.*, **23**, 674–683.
COSTALL, B. & NAYLOR, R. J. (1973d). *Arch. Pharmac.*, **278**, 117–133.
COSTALL, B. & NAYLOR, R. J. (1974a). *Eur. J. Pharmac.*, in the press.
COSTALL, B. & NAYLOR, R. J. (1974b). *Ibid.*, **25**, 121–129.
COSTALL, B. & NAYLOR, R. J. (1974c). *Neuropharmac.*, **13**, 353–364.
COSTALL, B. & NAYLOR, R. J. (1974d). *Arch. Pharmac.*, **285**, 83–91.
COSTALL, B. & NAYLOR, R. J. (1974e). *Eur. J. Pharmac.*, **27**, 46–58.
COSTALL, B. & NAYLOR, R. J. (1974f). *J. Pharm. Pharmac.*, **26**, 30–33.
COSTALL, B. & NAYLOR, R. J. (1974g). *Psychopharmacologia (Berl.)*, in the press.
COSTALL, B., NAYLOR, R. J. & PINDER, R. M. (1974). *J. Pharm. Pharmac.*, **26**, 753–762.
COSTALL, B., NAYLOR, R. J. & WRIGHT, T. (1972). *Arzneimittel-Forsch.*, **22**, 1178–1183.
FOG, R. (1972). *Acta neurol. scand., Suppl.* **50**, 1–66.
GRABOWSKA, M., ANTKIEWICZ, L. & MICHALUK, J. (1974). *J. Pharm. Pharmac.* **26**, 74–75.
MEDON, P. J., LEELING, J. L. & PHILLIPS, B. M. (1973). *Life Sci.*, **13**, 685–691.
RODRIGUEZ, R. & PARDO, E. G. (1971). *Psychopharmacologia (Berl.)*, **21**, 89–100.