The exact role of the dopamine stores in the above-mentioned feedback mechanism is not known. One may speculate that dopamine synthesis is regulated by the amount of the amine liberated from the stores. In consequence of blockade of dopaminergic receptors by chlorpromazine, a compensatory discharge of the amine might occur leading to an activation of its synthesis. Such an enhanced liberation of dopamine seems no longer possible if the stores have been emptied by reserpine. Consequently, the feedback mechanism may be impaired. An analogous regulatory mechanism possibly exists for the noradrenergic system, since neuroleptics are also known to block noradrenergic receptors.

In conclusion, experiments with various psychotropic drugs indicate that a relationship between disturbed extrapyramidal function and increased cerebral homovanillic acid levels seems to exist. The storage sites of dopamine may be involved in a feedback mechanism leading to an enhanced formation of homovanillic acid after blockage of dopaminergic receptors by neuroleptics.

Medical Research Department,
F. Hoffmann-La Roche & Co. Ltd.,
Basle, Switzerland.
July 15, 1966

M. DA PRADA
A. PLETSCHER

References

Andén, N.-E., Roos, B.-E. & Werdinius, B. (1963). *Life Sci.*, **2**, 448–458.
Andén, N.-E., Roos, B.-E. & Werdinius, B. (1964). *Ibid.*, **3**, 149–158.
Andén, N.-E., Carlsson, A., Dahlström, A., Fuxe, K., Hillarp, N.-Å. & Larsson, K. (1964). *Ibid.*, **3**, 523–530.
Burkard, W. P., Gey, K. F. & Pletscher, A. (1966). *Nature Lond.* In the press.
Carlsson, A. (1964). *Acta neuroveg.* **26**, 484–493.
Carlsson, A. & Lindqvist, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.
Carlsson, A. & Waldeck, B. (1958). *Acta physiol. scand.*, **44**, 293–298.
Gey, K. F. & Pletscher, A. (1964). *J. Pharmac. exp. Ther.*, **145**, 337–343.
Glowinski, J., Iversen, L. L. & Axelrod, J. (1966). *Ibid.*, **151**, 385–399.
Hornykiewicz, O. (1964). *Wien. klin. Wschr.*, **76**, 834.
Juorio, A. V., Sharman, D. F. & Trajkov, T. (1966). *Br. J. Pharmac. Chemother.*, **26**, 385–392.
Laverty, R. & Sharman, D. F. (1965). *Ibid.*, **24**, 759–772.
Pletscher, A., Burkard, W. P. & Gey, K. F. (1964). *Biochem. Pharmac.*, **13**, 385–390.
Da Prada, M. & Pletscher, A. (1966). *Experientia*, **22**, 465–466.
Roos, B.-E. (1965). *J. Pharm. Pharmac.*, **17**, 820–821.
Sourkes, T. L. (1961). *Rev. canad. Biol.*, **20**, 187–196.

The importance of the nervous impulse flow for the depletion of the monoamines from central neurones by some drugs

SIR,—It is known from previous work that the neuronal impulse flow is of great importance for the catecholamine depleting effect of α-methyl-*p*-tyrosine methylester (H 44/68), a potent and selective inhibitor of the enzyme tyrosine hydroxylase, since this drug causes a much more pronounced depletion of noradrenaline from the spinal cord cranial than caudal to a transection (Andén, Corrodi, Dahlström, Fuxe & Hökfelt, 1966). That study was based on the fact that all the 5-hydroxytryptamine (5-HT) and noradrenaline nerve terminals of the spinal cord belong to axons which originate from 5-HT and noradrenaline nerve cell bodies of the lower brain stem (Carlsson, Falck, Fuxe & Hillarp, 1964; Dahlström & Fuxe, 1965). Thus, after total transection of the spinal cord the nerve impulses will reach the monoamine nerve terminals lying cranial but not caudal to the lesion.

This neuronal model has now been utilised to test other drugs inhibiting the 5-HT as well as the catecholamine synthesis. These drugs are α-propyldopacetamide (H 22/54), a potent inhibitor of the 5-HT and catecholamine synthesis (Carlsson, Corrodi & Waldeck, 1963) and H 44/48, a selective inhibitor of the enzyme tryptophan hydroxylase. Furthermore, the effect of reserpine has also been studied, since this drug does not deplete the monoamine stores by synthesis inhibition but by blocking the uptake-storage mechanism of the amine granules (Carlsson, Hillarp & Waldeck, 1963; Dahlström, Fuxe & Hillarp, 1965).

Male, adult rats of 150–250 g have been used. The animals were acutely spinalised in the mid-thoracic region under ether anaesthesia. When the rats had woken up they were injected by the intraperitoneal route with the following drugs: H 22/54 (500 mg/kg, 4 hr before killing), H 44/48 (two doses of 500 mg/kg each, 3 and 1½ hr before killing) and reserpine (1 or 2·5 mg/kg, 4 hr before killing). The animals were kept in a temperature of +29° throughout the experiment to prevent the hypothermic action of the injected drugs.

For histochemical analysis the rats were killed by decapitation under light chloroform anaesthesia. Pieces of the spinal cord cranial and caudal to the site of transection were dissected out, freeze-dried, treated with formaldehyde gas, embedded in paraffin and mounted as previously described in detail (Dahlström & Fuxe, 1964; Hamberger, Malmfors & Sachs, 1965).

For biochemical analysis the rats were killed by a blow on the head. The spinal cord cranial and caudal to the lesion was taken out as quickly as possible. The pia mater and the roots were removed. For the 5-HT experiments the spinal cords from 2 or 3 rats were pooled. The noradrenaline and 5-HT were assayed spectrofluorimetrically after cation exchange chromatography (Häggendal, 1963; Andén & Magnusson, unpublished descriptions).

H 22/54. After injection of the drug H 22/54 to intact rats there was a marked and general decrease of 5-HT and noradrenaline in the 5-HT and noradrenaline nerve terminals of the whole spinal cord as revealed by both histochemical and biochemical determinations of these amines. When the drug was given after total transection, however, the same marked decrease of 5-HT and noradrenaline was observed in the respective nerve terminals cranial to the place of transection, whereas caudal to it both kinds of nerve terminals appeared to have practically normal amine levels. These findings were corroborated by biochemical experiments (Table 1). If an additional dose of H 22/54 was given 4 hr after the first dose the difference between the cranial and caudal half seemed to be even more pronounced.

H 44/48. After injection of H 44/48 to spinalized rats the intensity of the 5-HT nerve terminals as well as the biochemically determined 5-HT (Table 1) was reduced more in the cranial than in the caudal part, whereas in intact rats the decrease was about the same all over the spinal cord. The noradrenaline nerve terminals and the biochemically assayed noradrenaline were not changed.

Reserpine. After administration of reserpine to spinalized rats, with the doses used, there was a more pronounced depletion of the noradrenaline nerve terminals cranial than caudal to the lesion as revealed both histochemically and biochemically (Table 1). However, no significant difference was observed in the reductions of the amine levels of the 5-HT nerve terminals in the cranial and caudal part of the transected spinal cord (Table 1). In intact rats, reserpine produced a similar noradrenaline depletion in all parts of the spinal cord.

The present findings with H 44/48 and especially with H 22/54 show that the neuronal impulse flow is of great importance also for the depletion of 5-HT after inhibition of its synthesis. Thus, it is also possible to study the state of activity

in the 5-HT neurones with the help of potent inhibitors of the 5-HT synthesis in the same way as has been demonstrated for the catecholamine neurones.

TABLE 1. EFFECT OF CERTAIN DRUGS ON THE LEVELS (μg/g; MEAN $\pm$ S.E.) OF NORADRENALINE OR 5-HYDROXYTRYPTAMINE (5-HT) IN THE RAT SPINAL CORD CRANIAL AND CAUDAL TO A TOTAL TRANSECTION. The differences necessary for significance were calculated by analysis of variance. Number of experiments in parentheses.

Drug: dose and time	Amine	Part of the spinal cord	No drug treatment	Drug treatment	Reduction by drug %	Difference
H 22/54	5-HT	Cranial	0·39 ± 0·015(5)	0·18 ± 0·031(5)	54	50%
		Caudal	0·68 ± 0·065(5)	0·65 ± 0·061(5)	4	($P < 0·001$)
500 mg/kg, hr	Noradrenaline	Cranial	0·27 ± 0·029(3)	0·12 ± 0·029(3)	55	38%
		Caudal	0·48 ± 0·032(3)	0·40 ± 0·017(3)	17	($P < 0·025$)
H 44/48	5-HT	Cranial	0·33 ± 0·014(7)	0·17 ± 0·016(7)	47	23%
500 mg/kg × 2, 3 + 1½ hr		Caudal	0·74 ± 0·027(7)	0·57 ± 0·043(7)	24	($P < 0·01$)
Reserpine	Noradrenaline	Cranial	0·29 ± 0·015(6)	0·06 ± 0·007(6)	79	22%
		Caudal	0·42 ± 0·030(6)	0·18 ± 0·025(6)	57	($P < 0·01$)
2·5 mg/kg, 4 hr	5-HT	Cranial	0·24 ± 0·015(5)	0·10 ± 0·010(5)	59	−6%
		Caudal	0·56 ± 0·034(5)	0·20 ± 0·013(5)	65	

The results also show that the amine-depleting effect of reserpine is not dependent on the nervous impulse flow to the same high degree as that of synthesis inhibitors. Thus, the model used may also be of value to find out if an amine depleting drug acts by inhibiting the synthesis or by blocking the granule uptake.

Acknowledgements. This work has been supported by the Swedish State Medical Research Council (14X-502-02, 12X-714-02) and by Magnus Bergwall's Foundation. For generous gifts of drugs we thank Dr. H. Corrodi, AB Hässle, Göteborg (H 22/54, H 44/48) and CIBA Ltd., Stockholm (reserpine). For technical assistance we are indebted to Mrs. M. Baidins, Miss B. Lindberg and Miss B. Nilsdotter-Högberg.

Department of Pharmacology,
University of Göteborg.
N.-E. ANDÉN

Department of Histology,
Karolinska Institutet,
Stockholm,
Sweden.
F. FUXE
T. HÖKFELT

July 21, 1966

References

Andén, N.-E., Corrodi, H., Dahlström, A., Fuxe, K & Hökfelt, T. (1966). *Life Sci.*, **5**, 561–568.
Carlsson, A., Corrodi, H. & Waldeck, B. (1963). *Helv. chim. Acta*, **46**, 2270–2285.
Carlsson, A., Hillarp, N.-Å. & Waldeck, B. (1963). *Acta physiol. scand.*, **59**, *Suppl.* 215,1–38.
Carlsson, A., Falck, B., Fuxe, K. & Hillarp, N.-Å. (1964). *Ibid.*, **60**, 112–119.
Dahlström, A. & Fuxe, K. (1964). *Ibid.*, **62**, *Suppl.* 232, 1–55.
Dahlström, A. & Fuxe, K. (1965). *Ibid.*, **64**, *Suppl.* 247, 1–36.
Dahlström, A., Fuxe, K. & Hillarp, N.-Å. (1965). *Acta pharmac. tox.*, **22**, 277–292.
Hamberger, B., Malmfors, T. & Sachs, Ch. (1965). *J. Histochem. Cytochem.*, **13**, 147.
Häggendal, J. (1963). *Acta physiol. scand.*, **59**, 242–254.