Hydroxytryptamine turnover decreased by the antidepressant drug chlorimipramine

The antidepressant action of imipramine and related drugs has been ascribed largely to a blockade of one of the amine transporting mechanisms of the central monoamine containing neurons. This uptake mechanism, the membrane pump, is probably located at the level of the nerve cell membrane, and may form a major means of inactivating the neurotransmitter released into the synaptic area after nerve stimulation. The uptake mechanism and its blockade by imipramine-like agents have been studied extensively in noradrenaline-containing neurons, both centrally and peripherally (Andén, Carlsson & Häggendal, 1969). Recent experiments have revealed a similar uptake-concentration mechanism also in central 5-hydroxytryptamine (5-HT)-containing neurons. This membrane pump, too, is blocked by imipramine and, still more effectively, by chlorimipramine (Andén & others, 1969; Carlsson, Corrodi & others, 1969a; Carlsson, Jonason & others, 1969b). Imipramine has also been shown to slow the turnover rate of brain 5-HT (Corrodi & Fuxe, 1968; Schildkraut, Schanberg & others, 1969). Blockade of re-uptake may cause an increased amount of 5-HT to reach its receptors, or may cause the released 5-HT to remain near the receptors longer. It has been suggested that antidepressants cause an increase in receptor stimulation. Negative feedback mechanisms might then cause the impulse frequency of the 5-HT nerve to decrease, and thus lower the rate of 5-HT turnover. Since these antidepressant drugs may act partly by virtue of their influence on 5-HT neurons, it should be worthwhile to examine these alterations further.

Two methods have been used to examine the effects of antidepressants on 5-HT turnover. In the first, a tryptophan hydroxylase inhibitor was used to block 5-HT synthesis (Corrodi & Fuxe, 1968). Pretreatment with imipramine partly prevented the resulting 5-HT depletion. Therefore, it appeared that the rate of 5-HT breakdown or leakage was decreased. However, the correct turnover rates are difficult to calculate from such experiments, since decreasing the 5-HT concentration by synthesis inhibition may by itself slow the turnover. The second method involved intracerebral injection of labelled 5-HT and noting its disappearance (Meek, 1968; Schildkraut & others, 1969). In two separate investigations, imipramine slowed the disappearance of intracerebrally injected ^{14}C-5-HT. However, the injected amine may label only a small part of the endogenous pool, which might lead to erroneous conclusions.

A third approach takes advantage of the fact that there is only one detectable metabolite of 5-HT in brain, 5-hydroxyindoleacetic acid (5-HIAA). If no 5-HIAA can leave the brain, and no exogenous 5-HIAA can enter the brain, then the rate of 5-HT breakdown can be measured by determining the rate of accumulation of 5-HIAA. Probenecid appears to block the active efflux of 5-HIAA from brain (Sharman, 1966; Neff, Tozer & Brodie, 1967; Werdinius, 1967a; Diaz, Ngai & Costa, 1968). Neff & others (1967) have suggested that this blockade is complete, and that neither probenecid, nor the increased 5-HIAA level alters 5-HT metabolism. We have used this method to estimate the changes in 5-HT breakdown produced by chlorimipramine.

Male Sprague–Dawley rats, 150–200 g were injected intraperitoneally with chlorimipramine (15 mg/kg). Fifteen min later, they and control rats received probenecid (200 mg/kg, i.p.). At intervals, the animals were decapitated, brains from three rats pooled and analysed for 5-HIAA (Werdinius, 1967b). Four or five determinations were made at each interval for each of the two treatments.

Fig. 1 shows the effect of chlorimipramine on the accumulation of 5-HIAA. In animals treated only with probenecid, brain 5-HIAA levels increased linearly (0·22 μg/g h^{-1}) for 2 h. The animals pretreated with chlorimipramine accumulated

5-HIAA more slowly (0·068 μg/g h^{-1}). The difference in slopes (Davies, 1949) was statistically significant ($P<0{\cdot}005$).

The rate of accumulation of 5-HIAA after probenecid was lower than that reported by Diaz & others (1968) (0·29 μg/g h^{-1}) or by Neff & others (1967) (0·40 μg/g h^{-1}). A difference between strains of rats is one possible cause for this discrepancy. Brodie & others (1966) examined catecholamine turnover rates in rats from two different sources, and found one twice as fast as the other.

If the accumulation of 5-HIAA is to be a measure of 5-HT turnover, the 5-HT concentration must remain constant. In a separate series of experiments in this laboratory, chlorimipramine (15 or 25 mg/kg, i.p.) did not alter brain 5-HT levels.

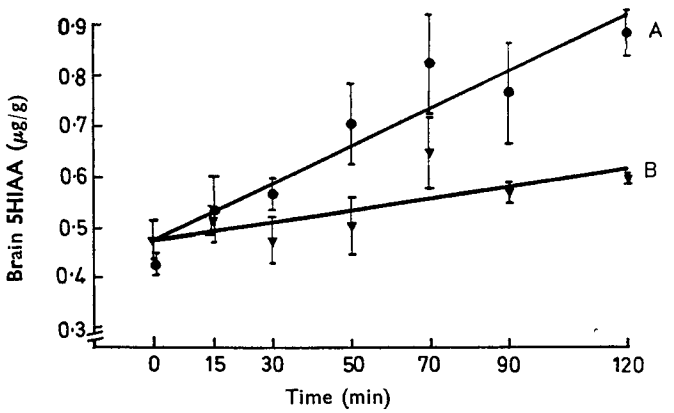

FIG. 1. Accumulation of 5-HIAA in rat brain after probenecid blockade of 5-HIAA efflux. Probenecid (200 mg/kg, i.p.) was injected at time zero to untreated controls, (A), or to animals pretreated with chlorimipramine (15 mg/kg, i.p.), 15 min earlier (B). The values represent mean ± s.e. of 4 or 5 determinations, each comprising 3 pooled whole brains.

From the present experiments, it seems likely that the 5-HT turnover rate of rats treated with chlorimipramine was only 30% of that of control animals. The cause of this effect on turnover is uncertain. Two possibilities are that chlorimipramine facilitated diffusion of 5-HIAA or antagonized the blockade of active efflux by probenecid. However, since two other independent methods show that imipramine-like drugs alter 5-HT turnover, it seems more likely that the change in 5-HIAA accumulation was a result of reduced the 5-HT oxidation. Possible explanations may be that 5-HT was prevented from reaching monoamine oxidase, or that rate of release of 5-HT declined as a consequence of some negative feed-back mechanism.

In addition to turnover studies, a variety of other techniques show that antidepressant drugs affect 5-HT in the central nervous system. For example, imipramine and chlorimipramine prevent depletion of brain 5-HT by the displacing agent, 4-methyl-α-ethyl-*m*-tyramine (Carlsson & others, 1969), probably by blocking the neuronal uptake of the displacing amine. Imipramine, but not desmethylimipramine, blocks uptake of intracerebrally injected 5-HT. There is also histochemical and *in vitro* evidence (Carlsson & others, 1969) to suggest that extraneuronal 5-HT concentration rises as a result of imipramine or chlorimipramine treatment.

Our findings lend further support to the possibility that antidepressant drugs may act, at least partly, by altering some effect of 5-HT in brain.

This investigation was supported by the Swedish Medical Research Council (B69–14X–2218–04, B69–60F–2670–01), the Medical Faculty, University of Göteborg, and Läkemedelsindustriföreningen. We acknowledge the assistance of Miss

Majbritt Hansson. We also thank J. R. Geigy AG for chlorimipramine, and Astra Ltd., Södertälje for probenecid.

Department of Pharmacology,
University of Göteborg,
Fack, S–400 33 Göteborg 33, Sweden.

JAMES MEEK
BENGT WERDINIUS

November 10, 1969

REFERENCES

ANDÉN, N.-E., CARLSSON, A. & HÄGGENDAL, J. (1969). *Ann. Rev. Pharmac.*, **9**, 119–134.

BRODIE, B. B., COSTA, E., DLABAC, A., NEFF, N. H. & SMOOKLER, H. H. (1966). *J. Pharmac. exp. Ther.*, **154**, 493–498.

CARLSSON, A., CORRODI, H., FUXE, K. & HÖKFELT, T. (1969a). *Europ. J. Pharmac.*, **5**, 357–372.

CARLSSON, A., JONASON, J., LINDQVIST, M. & FUXE, K. (1969b). *Brain Res.*, **12**, 456–460.

CORRODI, H. & FUXE, K. (1968). *J. Pharm. Pharmac.*, **20**, 230–231.

DAVIES, O. (1949). *Statistical Methods in Research and Production, p.* 152. London: Oliver and Boyd.

DIAZ, P. M., NGAI, S. H. & COSTA, E. (1968). *Adv. Pharmac.*, **6B**, 75–92.

MEEK, J. (1968). Ph.D. thesis, University of North Carolina, Chapel Hill.

NEFF, N., TOZER, T. & BRODIE, B. B. (1967). *J. Pharmac. exp. Ther.*, **158**, 214–218.

SCHILDKRAUT, J. J., SCHANBERG, S. M., BREESE, G. R. & KOPIN, I. J. (1969). *Biochem. Pharmac.* **18**, 1971–1978.

SHARMAN, D. F. (1966). *Br. J. Pharmac. Chemother.*, **28**, 153–163.

WERDINIUS, B. (1967a). *Acta pharmac. tox.*, **25**, 18–23.

WERDINIUS, B. (1967b). *Ibid.*, **25**, 1–8.

Prevention of experimental gastric ulcer in rats by a substance which increases biosynthesis of acid mucopolysaccharides

Biosynthesis of acid mucopolysaccharides, essential components of the connective tissue, has been much studied. The simplest approach is to investigate the incorporation of $^{35}SO_4$ into cartilage. The uptake of $^{35}SO_4$ is inhibited by steroid and non-steroid anti-inflammatory agents, both *in vitro* and *in vivo* in a dose-response relation (Bollet, 1961; Whitehouse & Boström, 1962; Szigeti, Ezer & others, 1965; Ezer & Boström, 1968).

ϵ-*p*-Chlorocarbobenzoxy-L-lysin-OMe-HCl (KL–11), increased the incorporation of $^{35}SO_4$ into the cartilage of the rat *in vivo* (Szporny, Ezer & others, 1969) and also prevented the inhibition of uptake of ^{35}S caused by prednisolone.

More and more importance is now attached to acid mucopolysaccharides that are present in large amounts in the gastric mucous membrane. Denko (1958) has shown that administration of hydrocortisone to hypophysectomized rats reduced the incorporation of ^{35}S into the tissues of the stomach. Kent & Allen (1966) have found that the $^{35}SO_4$ and glucose-U-^{14}C uptake by the gastric mucosa can be inhibited by sodium salicylate. It now seems equally certain that a significant inhibition of the synthesis of acid mucopolysaccharides can be achieved in the gastric mucous membrane by anti-inflammatory drugs. Perrey (1968) has described a parallel between the erosion of the gastric mucous membrane and the inhibition of glucosamine-6-phosphate synthesis by salicylate treatment. Since great importance is attached to the mucin content of the gastric mucous membrane in protecting the gastric wall against gastric juices, particularly hydrochloric acid, it seems that the damaging effect of anti-inflammatory substances in inhibiting the synthesis of mucopolysaccharides arises in this way.