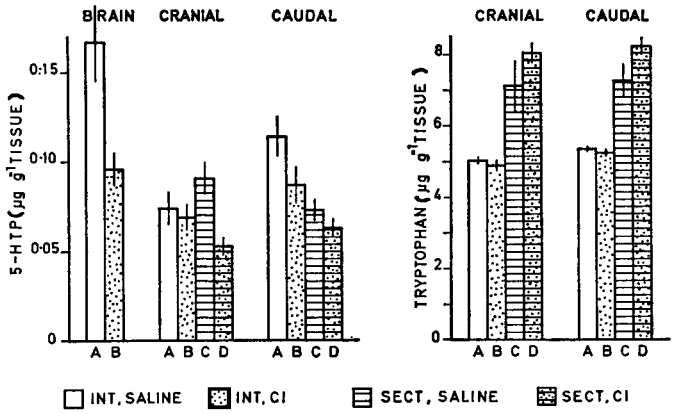
Effect of chlorimipramine on the rate of tryptophan hydroxylation in the intact and transected spinal cord

The antidepressant drug chlorimipramine has been shown to retard the turnover of brain 5-hydroxytryptamine (5-HT), when measured as the probenecid-induced accumulation of 5-hydroxyindoleacetic acid (5-HIAA) (Meek and Werdinius, 1970) or as the rate of ^{3}H-5-HT accumulation after intravenous injection of [^{3}H] tryptophan (Schubert, Nybäck & Sedvall, 1970). Evidence for a decreased 5-HT turnover is also reported after administration of the closely related compound imipramine (Corrodi & Fuxe, 1968; Schildkraut, Schanberg & others, 1969; Schubert & others, 1970; Bruinvels, 1972). Chlorimipramine and imipramine are potent inhibitors of the uptake mechanism for neurotransmittors at the cell membranes in central 5-HT-neurons (Carlsson, Corrodi & others, 1969a, b). This membrane pump is considered to be an important mechanism for the inactivation of released transmitters and inhibition of this mechanism is likely to result in an increased stimulation of 5-HT receptors (c.f. Meek, Fuxe & Andén, 1970; Modigh, 1973). Both drugs inhibit the impulse flow in central 5-HT neurons, possibly via a feed-back mechanism elicited by an increased activation of 5-HT receptors (Sheard, 1973). The reduction in 5-HT turnover, induced by the two drugs, has been suggested to be secondary to a reduction of the nerve impulse flow (Corrodi & Fuxe, 1968; Schubert & others, 1970). In line with this view, tryptophan hydroxylation, which normally is rate limiting in the 5-HT synthesis, appears to be to some extent influenced by the nerve impulse flow (Sheard & Aghajanian, 1968; Kuhar, Roth & Aghajanian, 1971; Shields & Eccleston, 1972; Carlsson, Lindqvist & others, 1973). Alternatively, the reduction in 5-HT turnover after administration of chlorimipramine or imipramine may be due to direct effects of the drugs on 5-HT metabolism. Such a mechanism has been emphasized by Bruinvels (1972) who found that imipramine inhibits the uptake of the 5-HT precursor L-tryptophan into synaptosomes.

In the present experiment the administration of chlorimipramine was found to retard the hydroxylation of tryptophan, estimated *in vivo* as the rate of accumulation of 5-hydroxytryptophan (5-HTP) after inhibition of aromatic amino-acid decarboxylase (DC) (for a discussion of this method, see Carlsson, Davis & others, 1972). A possible relation between this effect and changes in the nerve impulse flow was investigated by comparing the effect of chlorimipramine on the accumulation of 5-HTP, after DC inhibition, in the spinal cord below and above an acute transection. The 5-HT-containing neurons in the spinal cord have their cell bodies in the brain stem (Carlsson, Falck & others, 1964; Andén, Häggendal & others, 1964; Dahlström & Fuxe, 1965). Hence a spinal transection will block the flow of nerve impulses to the nerve terminals below the transection.

Male Sprague-Dawley rats, 180–220 g, were administered chlorimipramine in a dose of 15 mg kg^{-1} followed 30 min later by the DC inhibitor NSD 1015 (3-hydroxybenzylhydrazine HCl) (Carlsson & others, 1972) in a dose of 100 mg kg^{-1}. Control animals received 0·9% saline solution instead of chlorimipramine. The drugs were dissolved in 0·9% saline solution and injected intraperitoneally in a volume of 10 ml kg^{-1}. Half of the animals were spinalized at the midthoracic level under ether anaesthesia 2 h before the administration of chlorimipramine or 0·9% saline. The remainder received only ether anaesthesia at the corresponding time interval. All animals were exsanguinated under light chloroform anaesthesia 30 min after the administration of NSD 1015. Four whole brains or halves of the spinal cord were pooled for each biochemical determination and homogenized in 10 ml 0·4N perchloric



	BRAIN	CRANIAL	CAUDAL
A-B	P=0·01	N. S.	P<0·025
C-D	—	P<0·005	N. S.
A-C	—	N. S.	P<0·001
B-D	—	N. S.	P<0·05

	CRANIAL	CAUDAL
A-B	N. S.	N. S.
C-D	N. S.	N. S.
A-C	P<0·001	P<0·005
B-D	P<0·001	P<0·001

FIG. 1. Effect of chlorimipramine (Cl) on the concentrations of 5-hydroxytryptophan (5-HTP) and tryptophan in the brain and in the cranial and caudal part of the spinal cord of intact (INT) and spinally transected rats (SECT). Cl was given in a dose of 15 mg kg^{-1} i.p., followed 30 min later by NSD 1015, 100 mg kg^{-1} i.p. Control animals received 0·9% saline solution (SALINE) instead of Cl. The spinal transection was performed at the midthoracic level, 2 h before the administration of Cl or saline. All animals were killed 30 min after the administration of NSD 1015. The values are means of 6 (5-HTP) or 3 (tryptophan) determinations ± s.e. The data on each compound in the spinal cord were evaluated statistically by means of analysis of variance, followed by *t*-test (Winer, 1962). The brain data were analysed by means of *t*-test. $P > 0·05$ was considered not significant (N.S.).

acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. The extracts were purified by means of fractional elution on a strong cation exchange column (Dowex 50 W × 4, 12 mm × 4, 0 mm i.d.) (Atack & Magnusson, 1970; Kehr, Carlsson & Lindqvist, 1972) and analysed spectrophotofluorimetrically. 5-HTP and tryptophan were determined according to Bédard, Carlsson & Lindqvist (1972) and dihydroxyphenylanine (dopa) according to Kehr & others (1972).

Chlorimipramine, given to unoperated animals, significantly reduced the NSD 1015-induced accumulation of 5-HTP in the brain and in the caudal part of the spinal cord, but did not significantly affect the accumulation of 5-HTP in the cranial part of the spinal cord (Fig. 1). This unexpected behaviour of the cranial part requires further study and will not be commented upon here. In agreement with the observations of Carlsson & others (1973) transection of the spinal cord resulted in a reduction of the 5-HTP accumulation in the spinal cord below the transection but had no significant effect on the 5-HTP accumulation in the cranial part. Chlorimipramine, given to transected animals, reduced the 5-HTP accumulation in the cranial, but not in the caudal part of the spinal cord. The administration of chlorimipramine had no significant effect on the concentration of tryptophan in the spinal cords of either intact or transected NSD 1015-treated animals. In all transected animals tryptophan levels were higher than in unoperated animals in both parts of the spinal cord (*cf.* Carlsson & others, 1973). The NSD 1015-induced accumulation of dopa in the brains of unoperated animals was of the same magnitude in saline- and chlorimipramine-pretreated animals (means ± s.e.; 0·208 ± 0·018 and 0·201 ± 0·019 $\mu g\ g^{-1}$, respectively; $n = 6$).

The present observations support earlier findings obtained by means of different techniques that chlorimipramine has an inhibitory action on 5-HT synthesis and turnover in the central nervous system. The absence of such an effect below and its

persistence above an acute spinal transection is in line with the hypothesis that the effect is indirect and mediated *via* an action on the nerve impulse flow. However, further work is necessary to solve this problem.

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