Non-specific actions of dihydroxylated tryptamines in the central nervous system of the rat

It has been reported previously that 5,6-dihydroxytryptamine (5,6-DHT) produces axonal degeneration of central indoleamine neurons. This is evidenced by a long-lasting reduction of levels of 5-hydroxytryptamine (5-HT) in the brain and spinal cord, as well as fluorescence microscopic and ultrastructural signs of axonal degeneration (Baumgarten, Björklund & others, 1971; Baumgarten, Evetts & others, 1972). We have also reported previously biochemical evidence for a selective effect of 5,6-DHT (Regis), including decreases of endogenous concentrations of 5-HT, but not of noradrenaline (Baldessarini & Gerson, 1973). Also, there was a loss of ability of isolated nerve endings to take up low concentrations of labelled 5-HT, but not noradrenaline, and a corresponding failure of cns slices to release previously accumulated labelled 5-HT in the presence of 50 m mol litre^{-1} of K^+. Furthermore, the ability of isolated nerve endings *in vitro* or the intact rat cns *in vivo* to synthesize 5-HT and its major metabolite, 5-hydroxyindoleacetic acid, from labelled tryptophan was markedly decreased. However, recent studies indicate that 5,6-DHT is selective for indoleamine neurons only in a restricted dose-range, and that it can cause unspecific damage to catecholamine-containing neurons at intra-cranial doses above 75 μg (Baumgarten & Lachenmayer, 1972). It has been suggested that the analogous compound, 5,7-DHT, might be more useful for inducing an efficient and selective destruction of central indoleamine-neurons (Baumgarten & Lachenmayer, 1972). We have compared the effects of three dihydroxylated congeners: 5,6-, 5,7- and 6,7-DHT on concentrations of 5-HT and noradrenaline in the lower brainstem and spinal cord of the rat in an attempt to evaluate the relative specificity of these three substances.

The hydroxylated tryptamines were dissolved in a vehicle (artificial csf with ascorbic acid, 5 mg ml^{-1}, added to retard oxidation) and injected intracisternally in a volume of 25 μl into 180–200 g male Sprague-Dawley rats. Control animals were given the vehicle only. One week following this treatment the animals were killed and the medulla-spinal cord was removed and homogenized in 0·1 N HCl or 0·4 N perchloric acid for assays of 5-HT and noradrenaline, respectively. The extracts were prepared for ion-exchange chromatography on columns of Amberlite CG 50 (H^+) to isolate 5-HT (Diaz & Huttunen, 1972). The 5-HT was eluted with 3·5 N HCl and was estimated spectrophotofluorimetrically after conversion to the *o*-phthaldialdehyde derivative (Maickel & Miller, 1966). Noradrenaline levels were estimated by the trihydroxyindole fluorimetric assay following recovery by alumina column chromatography (Baldessarini, Lipinski & Chace, 1972). The methods are those described by Baldessarini & Gerson (1973).

Large decreases of endogenous 5-HT were produced by all three compounds (Table 1). The 5,6- and 5,7-isomers produced similar effects at a dose of 75 μg, while a smaller dose (25 μg) of the 6,7-isomer was as effective as 75 μg of the other two drugs. However, the latter substance (6,7-DHT) was highly toxic: about 30% of the animals given 25 μg of it failed to survive for a week, and the LD50 of the 6,7-compound was found to be about 50 μg. Endogenous levels of noradrenaline were not affected by 75 μg of 5,6-DHT (Table 1). In contrast, 75 μg of 5,7-DHT or 25 μg of 6,7-DHT reduced noradrenaline-levels by approximately 50% (Table 1). When rats were pretreated with desipramine (donated by Lakeside Laboratories), 25 mg (i.p.) 4 and 1 h before 100 μg of 5,7-DHT, noradrenaline was depleted by only 13 $\pm$ 1% ($P > 0{\cdot}10$).

These results are consistent with our previous findings (Baldessarini & Gerson, 1973) which suggested that 5,6-DHT at doses of 75 μg or less is a useful compound

Table 1. *Effects of dihydroxylated tryptamines on levels of serotonin (5-HT) and noradrenaline (NA).*

	Amine levels, ng g^{-1} ± s.e.			
	Controls	5,6-DHT (75 μg)	5,7-DHT (75 μg)	6,7-DHT (25 μg)
5-HT	457·8 ± 12·4 (100%)	257·2 ± 16·5* (56%)	208·9 ± 28·2* (46%)	212·5 ± 9·2* (46%)
NA	303·0 ± 19·0 (100%)	286·1 ± 16·9 (94%)	155·6 ± 8·3* (51%)	164·0 ± 15·3* (54%)

Rats (7 or more per group) were given one of the three dihydroxytryptamine analogues (DHT) or a vehicle intracisternally and killed a week later. Endogenous levels of 5-HT and noradrenaline (ng g^{-1} wet wt ± s.e.) in the medulla-spinal cord were assayed. (*): $P < 0{\cdot}001$ by *t*-test. The effect of 5,6-DHT on noradrenaline was not significant.

with which to produce efficient, selective destruction of central 5-HT-containing nerve terminals. The present results further suggest that the 5,7- and 6,7-analogues produce non-specific damage; including destruction of catecholamine-containing neurons in the lower brainstem and spinal cord, where the dihydroxytryptamines produce the greatest depletion of 5-HT (Baumgarten & Lachenmeyer, 1972). The 6,7-analogue appears to be more potent than the other compounds, which have similar potencies in our experiment. It might be possible to prevent the non-specific effects of 5,7-DHT, at least upon noradrenaline-containing neurons, by pretreatment with a drug (desipramine) which blocks uptake into those neurons. In conclusion, the 5,7- and 6,7-congeners of 5,6-DHT do not appear to offer advantages over the 5,6-compound as 5-HT-neuron destroying agents and may be disadvantageous in producing non-specific damage to neurons which do not contain indoleamines. A general limitation of the available dihydroxylated tryptamines continues to be their inability to produce profound depletion of 5-HT except in the spinal cord, even at doses which also induce non-specific destruction of other types of neurons.

This work was supported by U.S.P.H.S. (N.I.M.H.) Grant MH-16674. S.G. is a recipient of a training fellowship from a U.S. Public Health Service (N.I.M.H.) departmental grant: MH-07084 and an award from the Scottish Rite Research Fellowship Program, Supreme Council 33° A.A. Scottish Rite, Northern Masonic Jurisdiction of the U.S.A. R.J.B. is a recipient of a Research Scientist Development Award, National Institute of Mental Health: MH-74370. We are indebted to Dr. A. Manian of the Psychopharmacology Branch, NIMH for the generous gift 5,7-DHT and 6,7-DHT and to Ms. S. Wheeler for her excellent technical assistance. 5,6-DHT was obtained from Regis Co.

Psychiatric Research Laboratories,
Department of Psychiatry,
Massachusetts General Hospital
and Harvard Medical School,
Boston, Massachusetts 02114, U.S.A.

SYLVIA GERSON
ROSS J. BALDESSARINI

September 7, 1973

BALDESSARINI, R. J. & GERSON, S. (1973). *J. Pharm. Pharmac.*, **25**, 647–648.
BALDESSARINI, R. J., LIPINSKI, J. F. & CHACE, K. V. (1972). *Biochem. Pharmac.*, **21**, 77–87.
BAUMGARTEN, H. G., BJÖRKLUND, A., LACHENMAYER, L., NOBIN, A. & STENEVI, U. (1971). *Acta physiol. scand.* (*Suppl.* **373**), 1–16.

BAUMGARTEN, H. G., EVETTS, K. D., HOLMAN, R. B., IVERSEN, L. L., VOGT, M. & WILSON, G. (1972). *J. Neurochem.*, **19**, 1587–1597.

BAUMGARTEN, H. G. & LACHENMAYER, L. (1972). *Z. Zellforsch.*, **135**, 399–414.

DIAZ, J.-L. & HUTTUNEN, M. O. (1972). *Psychopharmacologia* (Berlin), **23**, 363–372.

MAICKEL, R. P. & MILLER, F. P. (1966). *Analyt. Chem.*, **38**, 1937–1938.

The dependence of imipramine-induced sedation upon central 5-hydroxytryptomine-like activity in the frog

Both 5-hydroxytryptamine-like and adrenaline-like actions of antidepressants are probably necessary for their therapeutic effect (Lapin & Oxenkrug, 1969). However, in the frog, the sedation and the typical twitches of the limbs caused by antidepressants like imipramine, most probably reflect the activation of the central 5-HT processes (Lapin & Oxenkrug, 1969). This hypothesis is based, not only on the minor role of noradrenaline in the frog brain, but also on the observations that the potentiation of the sedative effects of imipramine by reserpine or monoamine oxidase (MAO) inhibitors, or both, is related to an increase in "free brain 5-HT", which potentiation is blocked by 5-HT antagonists, while drugs influencing mainly the adrenergic processes are without any apparent behavioural effect in amphibians (e.g., chlorpromazine, amphetamine, AW-151129*) (Brodie, Bogdanski & Bonomi, 1964; Lapin, Oxenkrug & others, 1970; Oxenkrug & Lapin, 1971). Moreover, the rapid utilization of brain 5-HT *in vivo* (Harri, 1972a, b) and *in vitro* (Brodie & others, 1964) compared with that of adrenaline, supports the predominance of 5-HT processes in the brain of amphibians.

In the frog, the central 5-HT-like activity is greatly influenced by season and temperature acclimation (Harri, 1972a). Thus, it was of interest to study whether these changes are related to the sedative action of imipramine.

Frogs (*Rana temporaria*) were acclimatized to 5° (cold-acclimatized) and to 25° (warm-acclimatized) for at least 20 days before the experiments, in winter (February) and in summer (May). Imipramine, as aqueous solution, was injected into the dorsal lymph sac, and the onset of sedation (loss of the righting reflex) and its duration was recorded.

The durations of the sedation caused by a certain dose of imipramine were significantly longer in winter than in summer frogs ($P < 0{\cdot}001$; Mann-Whitney U-test) and also significantly longer in cold-acclimatized than warm-acclimatized animals in winter ($P < 0{\cdot}01$) but not in summer. The equipotent doses corresponding to the sedation for 4 h were 35 and 40 mg kg^{-1} for the winter frogs acclimatized to 5° and to 25°, and 54 and 57 mg kg^{-1} for the summer frogs, respectively. With these doses imipramine did not depress the limb movements or the pain and touch responses markedly. It only prevented the frogs from turning around on their limbs. This indicates that in spite of the high doses used the local anaesthetic action of imipramine is unimportant in influencing the results. On the other hand, chlorpromazine caused a loss of the righting reflex only with doses (over 150 mg kg^{-1}) which depressed all body movements and also were lethal.

The level of 5-HT was 1·93–3·06 $\mu g\ g^{-1}$ in the brain of winter frogs and 3·24–4·58 $\mu g\ g^{-1}$ in the summer frogs (Harri, 1972a). In the winter frogs, the level temporarily increased in warmth but was returned to the original level after 20 days, when the animals were used in experiments with imipramine.

When the 5-HT biosynthesis was inhibited with *p*-chlorophenylalanine (*p*CPA) (200 mg kg^{-1}) 2 h before killing the animals, its level in the brain was depleted by $1{\cdot}27 \pm 0{\cdot}042$ and $0{\cdot}89 \pm 0{\cdot}162\ \mu g\ g^{-1}$ in the cold acclimatized winter and summer

* 5-(*p*- Chlorphenyl)- 2,3,5,6, - tetrahydroimidazo (1, 2-c) quinazoline.