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LETTERS TO THE EDITOR

Effects of 5,6-dihydroxytryptamine on the metabolism of 5-hydroxytryptamine in the central nervous system of the rat

It has recently been reported that small amounts of 5,6-dihydroxytryptamine (DHT) injected into the cerebrospinal fluid (csf) can lead to prolonged selective decreases of 5-hydroxytryptamine (5-HT) in neurons of the central nervous system (cns) as determined chemically and histologically (Baumgarten, Lachenmayer & Schlossberger, 1972; Baumgarten, Evetts & others, 1972). We have investigated the effects of DHT on the synthesis, uptake and release of 5-HT in the cns of the rat.

DHT (calculated as free base) as the creatinine sulphate monohydrate salt (Regis) or its vehicle were injected intracisternally (i.c.) into 200 g male Sprague-Dawley rats in 20 μl of artificial csf with ascorbic acid added (5 mg ml^{-1}) as an antioxidant. In some experiments 5-HT levels were later measured by the ninhydrin-derivative fluorometric assay of Snyder, Axelrod & Zweig (1965) and noradrenaline levels were assayed by the trihydroxyindole fluorometric assay following recovery by alumina column chromatography (Baldessarini, Lipinski & Chace, 1972). In studies on uptake and release of 5-HT and noradrenaline tissues from rats pretreated with pargyline HCl (donated by Abbott Labs) (100 mg kg^{-1}, i.p.) were homogenized in isotonic sucrose and a crude synaptosomal fraction was prepared; tissues were incubated for 5 min with chromatographically pure [G-^{3}H]5-HT (17 Ci mmol^{-1}, Amersham/Searle) or (±)[7-^{3}H]noradrenaline (10 Ci mmol^{-1}, New England Nuclear) and synaptosomes were selectively recovered on Millipore filters and ^{3}H was counted (Baldessarini & Vogt, 1971). For release studies, minced tissues were similarly incubated, rinsed and then superfused with a physiologic buffer, followed by medium containing 50 mM K^+; serial fractions were collected and counted (Baldessarini & Vogt, 1972). The labelled material taken up and released was chromatographically identified as >90% unmetabolized labelled amine.

Other rats were given 20 μCi of L-[G-^{3}H]tryptophan (Trp) (1·2 Ci mmol^{-1}, New England Nuclear) with 100 μg of L-Trp (Sigma) intracisternally 15 min before death; the medulla-spinal cord was removed, homogenized in 0·1 N HCl containing antioxidants, centrifuged and fractionated on the following series of chromatographic

Table 1. *Effect of dihydroxytryptamine (DHT) on levels and uptake of 5-HT and noradrenaline.*

		5-HT		Noradrenaline	
		Content (ng g^{-1})	Uptake (nCi mg^{-1})	Content (ng g^{-1})	Uptake (nCi mg^{-1})
Medulla-spinal cord	Control	411±9	22·3±1·1	351±33	3·17±0·04
	DHT	217±13**	10·9±0·5**	333±37	3·28±0·05
Cerebral cortex	Control	277±16	42·7±1·1		
	DHT	197±27*	31·0±0·6**		
Subcortex-midbrain	Control	470±23	47·5±1·1		
	DHT	391±49	37·6±0·9**		

Rats (n≥6) were given 60 μg of DHT or its vehicle (i.c.) 1 week previously. Endogenous 5-HT or noradrenaline levels (ng g^{-1} wet tissue ± s.e.) and initial (5 min) uptake of 10^{-8} M [^{3}H]-5-HT or [^{3}H]noradrenaline into synaptosomes (nCi mg^{-1} protein ± s.e.) were measured. By *t*-test: * $P<0·05$; ** $P<0·001$.

columns: Amberlite CG 50 (H^+) to retain 5-HT, Sephadex G-10 to retain 5-hydroxyindole acetic acid (5-HIAA) and Dowex 50 (H^+) to retain Trp (Diaz & Huttunen, 1972) The columns were eluted and the radioactivity of each fraction was counted by liquid scintillation spectrometry.

DHT markedly decreased endogenous indoleamines, particularly in the lower brainstem and spinal cord and there were corresponding decreases in the ability of isolated nerve endings to transport [^{3}H]5-HT, while there was no effect on levels or uptake of noradrenaline (Table 1). The decreased uptake of 5-HT persisted for at least one month. There was a 75% decrease ($14{\cdot}8 \pm 1{\cdot}4$ *vs* $3{\cdot}8 \pm 0{\cdot}8$ nCi of [^{3}H]5-HT released above spontaneous efflux; mean $\pm$ s.e., n = 3, $P < 0{\cdot}005$), in the ability of minces of medulla-spinal cord to release [^{3}H]5-HT in the presence of 50 mM K^+, presumably a reflection of diminished uptake into 5-HT-neurons 1 week following 60 μg of DHT. When conversion of [^{3}H]Trp to 5-HT and its major metabolite was estimated *in vivo**, the amounts of labelled 5-HT and 5-HIAA recovered from medulla-spinal cord were decreased by 60–65%. At the same time, the radioactivity, content and specific radioactivity (Table 1) of Trp were not significantly changed. These results suggest that there was less synthesis of 5-HT from its precursor.

Thus, DHT introduced into the csf can produce large, persistent decreases in the concentrations of indoleamines in the mammalian cns, especially in the lower brainstem and spinal cord. The highly specific functions of high-affinity transport of labelled 5-HT, its release by depolarizing [K^+], and synthesis of 5-HT and 5-HIAA from labelled Trp were all decreased 1–4 weeks after DHT. These findings support the conclusion that DHT leads to severe, selective and long-lasting damage to central indoleamine-containing neurons, much as 6-OH-dopamine has been reported to do with catecholamine neurons.

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* By giving rats (n = 6) 75 μg of DMT or its vehicle (i.c.) 2 weeks earlier, killing them 15 min after i.c. injection of 20 μCi [^{3}H]Trp [s.a. $49{\cdot}4$ and $44{\cdot}3 \times 10^4$ d min^{-1} μg^{-1} for control and treated rats respectively], and separating metabolites from extracts of medulla-spinal cord, the control and DHT values (nCi g^{-1} tissues $\pm$ s.e.) for [^{3}H]5-HT and [^{3}H]5-HIAA were $10{\cdot}7 \pm 1{\cdot}2$ and $3{\cdot}7 \pm 0{\cdot}6$ $P < 0{\cdot}001$) and $9{\cdot}4 \pm 1{\cdot}2$ and $3{\cdot}9 \pm 0{\cdot}4$ $P < 0{\cdot}01$, respectively.