UPTAKE *IN VITRO* OF [^{14}C],5-HYDROXYTRYPTAMINE AND [^{14}C],5-HYDROXYTRYPTOPHAN BY REPRODUCTIVE TRACT TISSUES OF THE ADULT MALE RAT

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It has been reported that exogenous serotonin can interfere with both testicular exocrine and endocrine functions of the adult male rat (Boccabella, Salgado & Alger, 1962). These investigators postulated that the lesions may have resulted from testicular ischaemia since a vasodilator prevented the appearance of pycnotic primary spermatocytes and multinucleated spermatids induced by exogenous serotonin. More recently, Eccleston, Thoa & Axelrod (1968) have investigated the marked accumulation *in vitro* of [^{14}C]serotonin in guinea-pig ductus deferens. Previously, Taxi & Droz (1966) injected the serotonin precursor [^3H]5-hydroxytryptophan and demonstrated autoradiographically that the indoleamine accumulated in the sympathetic neurons of the rat ductus deferens.

The present study concerns the relative affinities *in vitro* of male rat reproductive tract tissues and kidney for [^{14}C],5-hydroxytryptamine ([^{14}C],5-HT) and its precursor [^{14}C],5-hydroxytryptophan ([^{14}C],5-HTP).

Mature, male Fisher rats (280 to 370 g) were maintained at 26° C and 14 hr light, with food and water available at all times. Animals were anaesthetized by intraperitoneal injection of sodium pentobarbitone and tissues were removed and sliced with a Stadie-Riggs microtome. Tissues from eight animals were weighed to the nearest 0.1 mg and 8- to 13-mg samples were put into 25-ml Erlenmeyer flasks containing the isotope with 4 ml Krebs-Ringer buffer (pH 7.4). The flasks were then placed in a shaking incubator at 37° C for 40 min under air. Incubations were done in triplicate.

The [^{14}C],5-HTP and [^{14}C],5-HT (New England Nuclear Corporation, 3.8 mCi/mol and 15.3 mCi/mmol, respectively) were diluted to 0.6 mg/ml for the stock solution. Aliquots of 1.5 ml stock solution were combined with 148.5 ml buffer to make the incubation medium. After incubation, the tissues were removed and rinsed twice for 5 min in fresh buffer.

The tissues were transferred to scintillation vials and 1 ml hyamine hydroxide (Packard Instrument Company) was added for digestion at 53° C for 4 hr. Vials were kept at room temperature for 36 hr before 0.5 ml benzoyl peroxide

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(1% in toluene) was added to each vial. After 10 min, 20 ml toluene-Liquifluor (New England Nuclear Corporation) was added. Vials were counted in a Packard Scintillation Spectrometer. Quenching was corrected by internal standardization using [^{14}C]toluene.

The data in Table 1 illustrate the profound difference in the apparent affinity of various segments of the male reproductive tract tissues for serotonin. Only vascular tissue accumulated significantly more [^{14}C],5-HT than kidney, although ductus deferens consistently demonstrated a relatively high affinity for this compound. In comparison, penile tissue and all three segments of the epididymis displayed very little ability to accumulate [^{14}C],5-HT. Testis parenchyma, tunica and seminal vesicle showed intermediate affinities for serotonin.

TABLE 1

UPTAKE *IN VITRO* OF [^{14}C],5-HYDROXYTRYPTAMINE AND [^{14}C],5-HYDROXYTRYPTOPHAN BY REPRODUCTIVE TRACT TISSUES OF THE ADULT MALE RAT

Tissue	Mean (d/min/mg/dry/wt)		S.D.		^{14}C uptake as a percentage of kidney value	
	[^{14}C],5-HT	[^{14}C],5-HTP	[^{14}C],5-HT	[^{14}C],5-HTP	[^{14}C],5-HT	[^{14}C],5-HTP
Kidney	6659	1234	1494	233	100	100
Pampiniform plexus- testicular artery	10846*	637†	1584	160	163	52
Ductus deferens	7588	637†	631	65	114	52
Seminal vesicle	6251	1813	587	698	94	147
Tunica albuginea	5779	865*	1119	287	87	70
Testis parenchyma	4605	4821	1123	434	69	391
Cauda epididymidis	3512	1525	897	382	53	124
Caput epididymidis	2627†	779	686	127	39	63
Corpus epididymidis	2592†	991	476	312	39	80
Penis	2146†	666	424	211	32	54

(Freund, Livermore & Miller, 1962).

*F statistic, significantly higher than value for kidney ($P < 0.05$).

†F statistic, significantly lower than value for kidney ($P < 0.05$).

When samples from these same tissues were incubated in the serotonin precursor [^{14}C],5-HTP, a very different pattern emerged (Table 1). Testis parenchyma, in which a very modest uptake of [^{14}C],5-HT had been demonstrated, accumulated [^{14}C],5-HTP nearly four times more efficiently than kidney and more than seven times more effectively than ductus deferens, vascular tissue or penis. Only seminal vesicle and cauda epididymidis were found to have a moderately high uptake of [^{14}C],5-HTP. Testis tunica and epididymal segments demonstrated intermediate affinities for this indoleamine.

Generally, those tissues which accumulated large amounts of serotonin

demonstrated very limited uptake of its precursor [^{14}C],5-HTP and vice versa. These findings are in agreement with those of Kormano & Penttilä (1969) who found that serotonin was a more potent stimulus for vasoconstriction than 5-HTP, but that 5-HTP accumulated in testis parenchyma more extensively than serotonin. We have presented data showing that testis parenchyma preferentially accumulated [^{14}C],5-HTP *in vitro* rather than serotonin, and that the vasculature supplying the testis selectively accumulated [^{14}C],5-HT as compared with its precursor. The preferential uptake of either indoleamine by these male reproductive tract tissues may reside with their inherent density of sympathetic neurons. Eccleston *et al.* (1968) have demonstrated the sub-cellular storage of serotonin in the sympathetic neurons of guinea-pig ductus deferens after incubation *in vitro*. However, Bogdanski, Tissari & Brodie (1970), in studies on rabbit synaptosomes, reported that the mechanism of transport and storage of biogenic amines, especially serotonin, was stimulated by sodium ions and impaired by potassium ions. The data from the present investigation do not clarify the mechanism by which either [^{14}C],5-HT or [^{14}C],5-HTP accumulated in the various tissues tested. The uptake of these indoleamines, as well as their retention or release, could be regulated by enzymatic, ionic, or physical membrane properties.

Subsequent studies on the localization of these indoleamines in tissues of the male reproductive tract may lead to the mechanisms of the selective uptake observed in this study and clarify the rôle of biogenic amines in male reproduction.

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