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Spontaneous release of 5-hydroxytryptamine by brain slices

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INTRODUCTION AND METHODS

THIS report describes the results of some initial work on neurohumoral release from isolated brain slices and is specifically concerned with spontaneous release of 5-hydroxytryptamine (5-HT). We have used an approach analogous to that employed originally by Quastel *et al.* for acetylcholine; namely, the analysis of the saline medium in which brain slices have incubated in the presence of an enzyme inhibitor that prevents destruction of the neurohumour.[1]

Male white rats weighing approximately 125 g were decapitated and the brains rapidly removed. Horizontal or sagittal slices of whole brain, 0·5–0·7 mm thick, were made with 40-gauge silver wire mounted on a plastic frame.[2] Usually three slices per incubation flask were used. Flasks contained 2·3–4·8 ml of a modified Tyrode's solution, pH7·2, with the following composition: NaCl, 0·098 M; KCl, 0·0027 M; $CaCl_2$, 0·0012 M; $MgCl_2$, 0·0013 M; KH_2PO_4, 0·0004 M; glucose, 0·01 M; $NaHCO_3$, 0·025 M. We found that 0·1–0·2 ml l-phenyl-2-hydrazinopropane (JB-516, 10^{-3} M) was necessary for the consistent appearance of 5-HT in the incubation medium. Some experiments were carried out in sealed flasks with 95% O_2–5% CO_2 aeration; others were performed in open flasks. Incubations were for 1–3 hr at 37° with gentle shaking, after which the incubation saline was decanted and assayed for 5-HT or stored at −10° until assayed. Slices were blotted on filter paper, weighed, and extracted for bioassay of 5-HT by the method of Amin *et al.*[3] In addition, several extractions were carried out with incubation saline pooled from 3 to 4 flasks for purposes of fluorometric identification of 5-HT.[4,5] Owing to the small quantities of 5-HT present in the pooled incubates, spectra obtained were resolved[6] with the repetitive analog computer.* Bioassay of the filtrates and extracted slices on the isolated heart of *Venus mercenaria* for 5-HT activity was carried out as described by Welsh.[7] Contractions were recorded on inkwriting kymograph or polygraph. Benzoquinonium chloride (Mytolon, 10^{-5} g/ml) was present in the seawater to block any acetylcholine effect.[8,9] For the assay, two dilutions of standard were matched with two dilutions of unknown; 5-HT activity was verified by use of the 2-bromolysergic acid diethylamide (BOL-148, 10^{-5} M), a specific 5-HT antagonist on the clam heart.[10] Incubation saline containing JB-516 and/or reserpine in the concentrations used in the incubations was used as a control and failed to affect the properly prepared assay preparation. Standard 5-HT solutions were prepared from the creatinine sulfate. Reserpine (10^{-5} g/ml) was used as the phosphate, and all other compounds refer to the salt.

TABLE 1. 5-HT CONTENT OF THE INCUBATE AND BRAIN SLICES AFTER INCUBATION OF RAT WHOLE-BRAIN SLICES IN A MODIFIED TYRODE'S SOLUTION*

Condition	Incubate	Brain slices
Unincubated slices		0·624 (0·472 – 0·880; 4)
Incubation 37°, JB-516 (5×10^{-5} M) only	0·166 ± 0·010 (15)	0·443 (0·372 – 0·490; 4)
Incubation 37°, JB-516 and reserpine (10^{-5} g/ml)	0·377 ± 0·054 (8)	0·288 (0·213 – 0·360; 4)

* Values are expressed as mean micrograms 5-HT creatinine sulfate per g wet slices per 2-hr incubation, followed by standard error of mean or range and number of experiments.

RESULTS AND DISCUSSION

Using horizontal slices of the anterior half of the brain we found that incubates from flasks with JB-516 only contained less 5-HT activity (0·166 ± 0·010 μg 5-HT creatinine sulfate/g wet slices/2 hr; 15 experiments) than incubates from flasks with JB-516 and reserpine (0·377 ± 0·054; 8 experiments, Table 1). We obtained essentially the same results when each flask contained three sagittal whole-

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brain slices, beginning at the medial surface of a hemisphere. Thus the differences are not explainable in terms of regional variations in 5-HT concentration. Therefore reserpine added to slices *in vitro* seems to cause an initial increase in spontaneous 5-HT release. After incubation, slices from flasks containing only JB-516 measured 0·443 (0·372-0·490, 4 values) μg 5-HT creatine sulfate/g wet slices. Slices from flasks containing reserpine in addition measured 0·288 (0·213–0·360, 4 values). Unincubated slices rinsed in the incubation medium for 5 min and then extracted contained 0·624 (0·472–0·880, 4 values). In several experiments it was not possible to account for the 5-HT activity of the incubate by ascribing it to loss of original 5-HT from the slice. That is to say, the total (incubate plus slice) 5-HT was sometimes greater than the average 5-HT content of the unincubated slice. Thus the question of synthesis and/or release from bound stores as an explanation of the 5-HT activity in the incubate has not been settled at this point. Oxygenation of the flasks seemed to lower the 5-HT activity of the incubates, higher values being obtained when incubation was performed in open flasks. Fluorescence spectra obtained as described above confirmed the presence of 5-HT in the incubate, but the amounts were too small to permit accurate measurement.

Thus whole-brain slices release 5-HT spontaneously *in vitro*, and this release can be initially accelerated by adding reserpine to the medium. At this point our studies do not settle the issue of the origin of the 5-HT released, whether it be from bound stores only or also from synthesis. A recent report by Andén *et al.* suggests that 5-HT synthesis may occur in isolated frog and rat spinal cord during electrical stimulation.[11]

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Epoxidation of aldrin, isodrin, and heptachlor by rat liver microsomes

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THE CONVERSION of aldrin, isodrin, and heptachlor to their respective epoxides* in soil and in plant and animal tissues is well known, the first report being that of Radomski and Davidow.[1] The epoxides

* Aldrin: 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-endoexo-dimethanonaphthalene.
Dieldrin: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a5,6,7,8,,8a-octa-hydro-1,4,5,8-endoexo-dimethanonaphthalene.
Endrin: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octa-hydro-1,4,5,8-endo,endo-dinonaphthalene.
Heptachlor: 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene.
Heptachlor epoxide: 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene.
Isodrin: 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-endo-endo-dimethanonaphthalene.