EFFECT OF RESERPINE, OUABAIN AND
p-CHLOROMERCURIBENZOATE ON THE RELEASE
OF 5-HYDROXYTRYPTAMINE FROM RAT
BRAIN SYNAPTOSOMES

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Accepted September 8, 1977

We found that when rat brain synaptosomes were pre-loaded with [^3H]-5-hydroxy-tryptamine ([^3H]-5-HT) and further incubated with 60 mM KCl, [^3H]-5-HT was released from the preparation (1). Furthermore, we obtained evidence which suggested that Na^+ and Ca^{2+} are requirements for 5-HT release but microfilaments or contractile protein do not participate in the release mechanism (1). To obtain further information, particularly with respect to ATPase activity, we studied the effect of reserpine, ouabain and p-chloromercuribenzoate (PCMB) on the release of 5-HT from synaptosomes, in vitro.

5-Hydroxy[G- ^3H]-tryptamine creatinine sulfate ([^3H]-5-HT) (500 mCi/mmole) was obtained from the Radiochemical Centre, Amersham. Reserpine was purchased from Nakarai Chemicals Ltd. (Kyoto, Japan); Ouabain from E. Merck, Japan Ltd. (Tokyo, Japan); PCMB from Katayama Chemical Industry Ltd. (Osaka, Japan).

Male Wistar strain rats (100–140 g) were decapitated and the whole brain (excluding cerebellum) was rapidly removed and dissected. The crude synaptosomal S_1 -fraction was prepared by the technique of Segawa et al. (2). Uptake of [^3H]-5-HT into S_1 -fraction was performed by the modified method of Nomura et al. (3) and Nomura et al. (4). At the end of incubation period, the reaction mixture was centrifuged at 11,500 g for 20 min and after twice washing the crude mitochondrial P_2 fraction it was suspended in Krebs-Ringer solution to yield a final protein concentration of 3.0–3.6 mg/ml. Release of [^3H]-5-HT from P_2 -fraction was performed by the method of Segawa et al. (1). After pre-incubation for 20 min at 30°C with 0.1 ml of 0.015 M Tris-buffered saline or test drugs dissolved in this solution, the samples were further incubated for 10 min with 60 mM KCl. After centrifugation at 11,500 g for 20 min, the radioactivity in the supernatant and the pellet was measured in a model 3320 Packard Tri-Carb liquid scintillation spectrometer and corrected for efficiency by external standardization. Release of [^3H]-5-HT was expressed as percent of the total tritium uptake. Potassium-induced release of [^3H]-5-HT was calculated by subtracting the estimated spontaneous [^3H]-5-HT release (the release in normal Krebs-Ringer solution) from the total [^3H]-5-HT release during incubation with high potassium. In 5 experiments, the released tritium was examined for the presence of metabolites of [^3H]-5-HT and it was found that the proportion of unchanged [^3H]-5-HT was 63.5% of the total release

of radioactivity in spontaneous release, while such was 82.1 % in potassium-induced release. ATPase activity was measured by determination of Pi release from ATP according to the method of Marsh (5). The protein was estimated by the Folin reagent method (6).

Initial rate of release was very rapid. Thus, within 5 min after incubation, more than 30 % of the radioactivity taken up during incubation was released. Thereafter, continuous slow release was observed for 30 min. Incubation with 60 mM KCl increased the tritium release from synaptosomes and the difference between these two releases is referred to as potassium-induced release.

As is shown in Table 1, reserpine (10^{-6} M) increased both the spontaneous and the potassium-induced release, though the increase in the spontaneous release was non-significant. Ouabain (10^{-5} M) did not affect either the spontaneous or the potassium-induced release but at 10^{-4} M this compound increased the spontaneous release and attenuated the potassium-induced release. As ouabain does inhibit synaptosomal Na^+ , K^+ -ATPase at 10^{-5} M (7), there was some doubt about the direct involvement of Na^+ , K^+ -ATPase in the release of 5-HT from synaptosomes. There are at least two possible explanations for the increase of the spontaneous release by ouabain. First, as has been reported (8-10), ouabain directly increased the influx of external Ca^{2+} . Second, reuptake mechanism of 5-HT was impaired by ouabain. The attenuation of the potassium-induced release by ouabain is partly due to the marked increase in the spontaneous release. PCMB did not affect either the spontaneous or the potassium-induced release at 10^{-5} M but at 10^{-4} M, markedly

TABLE 1. Effect of reserpine, ouabain and PCMB on the spontaneous and potassium-induced release of [^3H]-5-HT from rat brain synaptosomes

Drugs	Concentration (M)	Release	
		Spontaneous	Potassium-induced
None		25.9 $\pm$ 2.4 (3)	5.4 $\pm$ 0.4 (3)
Reserpine	10^{-6}	28.9 $\pm$ 2.1 (3)	8.3 $\pm$ 0.3 (3)**
Ouabain	10^{-5}	28.7 $\pm$ 2.2 (3)	5.7 $\pm$ 0.3 (3)
	10^{-4}	37.1 $\pm$ 3.3 (3)*	2.2 $\pm$ 0.7 (3)*
PCMB	10^{-5}	21.4 (2)	9.6 (2)
	10^{-4}	58.5 (2)	-2.1 (2)

Rat brain synaptosomes, pre-loaded with [^3H]-5-HT were incubated for 10 min in Krebs-Ringer solution containing drugs. Potassium-induced release is expressed as excess of spontaneous release during incubation in high potassium medium. Values are expressed as mean % of total tritium uptake $\pm$ S.E.M. Figures in parentheses are the number of determinations. * $P < 0.05$, ** $P < 0.01$.

TABLE 2. Effect of PCMB on ATPase activity of rat brain synaptosomes

Drug	Concentration (M)	ATPase activity (% of control)		
		Na^+ , K^+ -ATPase	Mg^{2+} -ATPase	Ca^{2+} -ATPase
PCMB	10^{-5}	54.7 (2)*	54.7 (2)*	62.9 (2)*
	10^{-4}	28.9 $\pm$ 5.3 (4)**	42.2 $\pm$ 2.7 (4)**	52.5 $\pm$ 0.8 (4)**

Figures in parentheses are the number of determinations. * $P < 0.05$, ** $P < 0.01$.

increased the spontaneous release and decreased the potassium-induced release (Table 1). PCMB inhibited significantly Na^+ , K^+ , Mg^{2+} - and Ca^{2+} -ATPase at 10^{-5} M (Table 2). Therefore, the concentration required to affect the 5-HT release was one order higher than that required to inhibit ATPase. This implies that PCMB affects 5-HT release through its effect on a cellular function other than ATPase.

Acknowledgement: This work was supported in part by grant No. 137014 for the research of science from the Department of Education, Science and Culture, Japan.

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POTENTIATION OF TYRAMINE ACTION BY 5-HYDROXYTRYPTAMINE IN THE DOG HEART

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Accepted September 16, 1977

We have already found that 5-hydroxytryptamine (5-HT) readily causes a release of norepinephrine by its tyramine-like action (1), as determined using the blood-perfused dog atrium and ventricular preparations (2-4). In the present study, we investigated the interaction between 5-HT and tyramine using the isolated atrial and ventricular preparations.

Seven adult mongrel dogs of either sex weighing 12 to 17 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The right atrial muscle was quickly removed and