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SHORT COMMUNICATION

Inhibition of Sodium- and Potassium-Dependent Adenosine Triphosphatase by Serotonin

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SUMMARY

GARCÍA-CASTIÑEIRAS, SIXTO, WHITE, JOHN I. & TORO-GOYCO, EFRAÍN (1977) Inhibition of sodium- and potassium-dependent adenosine triphosphatase by serotonin. *Mol. Pharmacol.*, 13, 181-184.

Serotonin was identified as the potent ($Na^+ + K^+$)-ATPase inhibitor previously reported to be present in the coelenterate *Palythoa caribaeorum*. As the serotonin–creatinine sulfate complex (commercial preparations) its inhibitory potency is sharply reduced. Inhibition is fully restored on fractionation of the complex on cation-exchange resins.

In previous studies conducted with crude preparations of palytoxin, a powerful marine toxin produced by several species of soft coral of the genus *Palythoa* (1), strong inhibitory effects on the ouabain-sensitive ($Na^+ + K^+$)-ATPase isolated from the electric organ of *Electrophorus electricus* were detected. Separation of the main toxin component of palytoxin, characterized by a strong contractile effect on smooth muscle, from the ATPase inhibitor was accomplished. It was shown that the latter was a small molecule that had the properties of an indole (2). We report here that the indole is serotonin and that the creatinine sulfate added as stabilizer to commercial preparations of serotonin markedly diminishes its inhibitory action.

The ($Na^+ + K^+$)-ATPase inhibitor was prepared as described previously (2). Several criteria were used to establish its identity with serotonin. Its chromatographic properties were studied by ascending paper chromatography on Whatman No. 3MM paper and by thin-layer chromatography using silica gel (Kontes Quantum) plates and polyethyleneimine-cellulose (Brinkmann) plates. The former plates were developed in 2-propanol–ethyl acetate–28% ammonia–dimethylformamide (35:45:20:5), and the latter, in 0.5 M (5 min), 1.0 M (10 min), and 1.5 M (30 min) LiCl (3). After drying, the plates were stained with Sprince's (4) or Procházka's (5) reagents. High-voltage electrophoresis was conducted at 60 mamp for 60 min in a Shandon apparatus using Whatman No. 3MM paper. A water–acetic acid mixture (15:1) adjusted to pH 4.0 with pyridine was used as solvent. All chromatographic work was performed using indoles of known structure for comparison (Table 1). Spectrophotofluorometric tests showed that when dissolved in 3 M HCl and excited at 300 nm, the inhibitor shifted its emission peak from 338 nm to 535 nm. Only serotonin exhibited this behavior.

The ($Na^+ + K^+$)-ATPase was isolated from the electric organ of *Electrophorus electricus* as described by Albers *et al.* (6), except that disruption of the tissue was

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TABLE 1

Chromatographic, electrophoretic, and spectrophotofluorometric evidence for identity of serotonin and ATPase inhibitor from P. caribaeorum

Compound	Chromatography			High-voltage electrophoresis	Emission in H_2O[a]	Emission in 3 M HCl[a]
	Paper	Thin-layer silica gel	Polyethylimine			
	R_F	R_F	R_F	*mm*	*nm*	*nm*
Indoleacetic acid	0.95	0.45				
5-Hydroxyindoleacetic acid		0.34		5		
5-Methoxyindoleacetic acid		0.43				
5-Hydroxytryptamine	0.52	0.81	0.37	84	338	535
6-Hydroxytryptamine		0.84		82	353	—[b]
5-Methoxytryptamine		0.88		85	—[c]	
N-Acetyl-5-hydroxytryptamine		0.95		16		
Tryptophan	0.54	0.39		17		
5-Hydroxytryptophan		0.30		15		
Inhibitor	0.52	0.82	0.37	84	338	535

[a] Excitation at 300 nm.
[b] No shift.
[c] No peak.

carried out in the presence of 5 mM EDTA by repeated homogenization. The preparations were 95% ouabain-sensitive, with a specific activity of 1.1 μM P_i per minute per milligram of protein.

Figure 1 illustrates the inhibitory activity of the purified ($Na^+ + K^+$)-ATPase inhibitor compared with that of commercial serotonin, which comes as a serotonin–creatinine sulfate complex. The complex approaches a plateau at 50% inhibition.

The unexpected finding of a diminished inhibitory effect of serotonin in its complex form led us to attempt to isolate serotonin from creatinine by cation-exchange chromatography, taking advantage of the differences in their charge properties. The separation of the two chemical entities is shown in Fig. 2.

The inhibitory activity shown by the serotonin so isolated is similar (on a weight basis) to that shown by the ATPase inhibitor isolated from extracts of *Palythoa caribaeorum* (Fig. 3).

A plausible explanation for the different ATPase-inhibitory activities of the serotonin–creatinine sulfate complex and the free compound may be based on purely structural considerations. The serotonin–creatinine sulfate complex exhibits an intricate pattern of ionic and hydrogen bonding (11) which is established indirectly between serotonin and creatinine through the sulfate. In addition, the indolic moiety of serotonin and the creatinine molecule are planar structures, a fact that facilitates their aggregation. Therefore it is reasonable to conclude that the binding of serotonin to any other chemical entity—e.g., a biological membrane, an active site of an enzyme, or a synaptic receptor—will be considerably diminished in the presence of creatinine sulfate. With this consideration in mind, the results shown by Figs. 2 and 3 should be expected. The same reasoning makes it advisable to repeat a number of experiments so far performed with the creatinine sulfate complex using instead serotonin purified as described above.

Recently Canessa *et al.* (12) reported inhibitory effects on ($Na^+ + K^+$)-ATPase by

several indolic compounds such as harmaline, harmalol, harmine, and lysergic acid diethylamide, but not by serotonin. Yoshimura (13) reported enhancement of brain ($Na^+ + K^+$)-ATPase activities by the serotonin–creatinine sulfate complex. However, his enzyme preparation was very crude and had not been treated with EDTA to remove chelating agents. The complex itself may act as a chelating agent, thus artificially enhancing enzyme activity. Kuriaki and Baba (14) found that serotonin inhibited ATPase activity from rat kidney. From their description, their enzyme probably represented a Ca^{++}-stimulated ATPase.

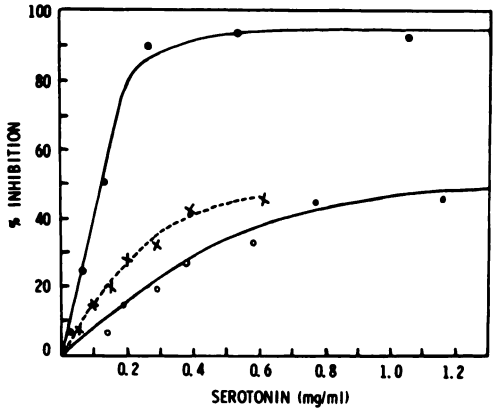

FIG. 1. *Electroplax ATPase inhibition by purified inhibitor from P. caribaeorum and serotonin–creatinine sulfate complex*

The standard assay medium contained Na^+, 150 mM; K^+, 20 mM; Mg^{++}, 5 mM; Cl^-, 180 mM; and Tris buffer, pH 7.2, 30 mM. After addition of the desired amount of enzyme and equilibration at 37° for 10 min, 2.5 mM ATP was added. The total volume of the incubation mixture was 1.0 ml. Incubation was performed for 20 min, during which the activity was linear. P_i was determined by the method of Baginski *et al.* (7), and protein, by the method of Lowry *et al.* (8). O——O, serotonin-creatinine sulfate complex; ×- - -×, inhibition corrected for creatinine sulfate content; ●——●, purified inhibitor from *P. caribaeorum*.

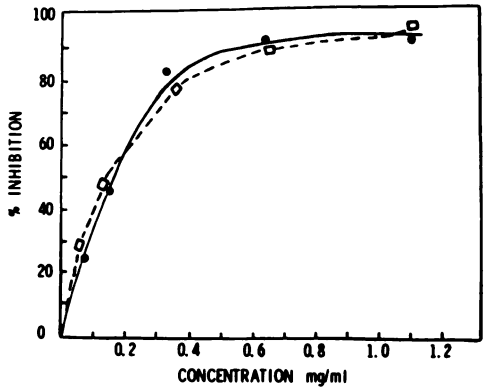

FIG. 3. *Electroplax ATPase inhibition by free serotonin obtained by carboxymethyl cellulose chromatography of serotonin–creatinine sulfate complex* (●——●) *and by inhibitor purified from extracts of P. caribaeorum* (□- - -□).

Assays were performed as described for Fig. 1.

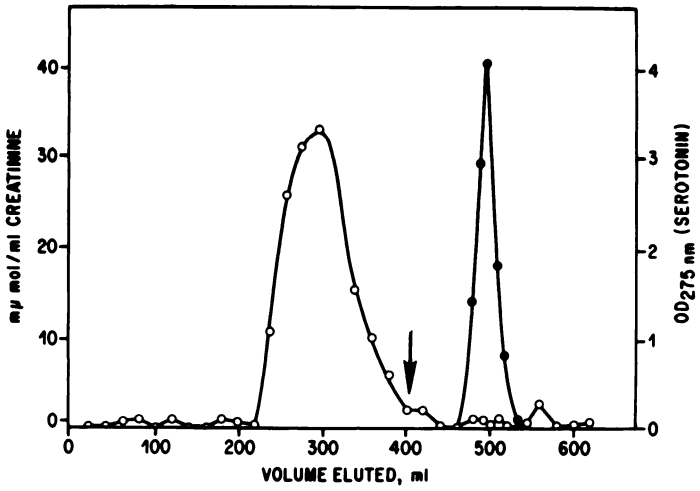

FIG. 2. *Fractionation of serotonin–creatinine sulfate complex on carboxymethyl cellulose columns*

Water adjusted to pH 4.0 with 1 mM HCl was used as eluent (first 400 ml), followed by 0.9% NaCl. Serotonin in the eluate (●——●) was monitored at 275 nm, using a UA-2 Isco ultraviolet monitor. E_{mM} for serotonin was taken as 5.0 (9). Creatinine (O——O) was determined by the method of DiGiorgio (10).

In this work we used a 0.5 mM concentration of serotonin to cause 50% inhibition of ATPase activity (Fig. 2). Serotonin concentrations of this order of magnitude are only found in intestinal (0.15 mM) and pineal gland (over 1 mM) tissues (15, 16). Serotonin is not evenly distributed throughout the tissues, but is found in vesicles, probably increasing its effective concentrations in specific locations of the cells.

The observations reported here may explain some of the physiological and pharmacological actions of serotonin and suggest that these actions should be reinvestigated using the free compound rather than commercial complexes.

Preliminary work (data not shown) indicates that serotonin may act by inhibiting the K^+-dependent dephosphorylation step in ATP hydrolysis (17). When *p*-nitrophenyl phosphate is used as substrate with our ATPase preparations, 1 mM concentrations of serotonin totally abolish the phosphatase activity. The mechanism of (Na^+ + K^+)-ATPase inhibition by serotonin is the object of further investigations.

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