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THE EFFECT OF ARIDANIN ISOLATED FROM *TETRAPLEURA TETRAPTERA* AND SEROTONIN ON THE ISOLATED GASTROINTESTINAL TRACT SMOOTH MUSCLES OF *BIOMPHALARIA GLABRATA* AND UPTAKE OF CALCIUM

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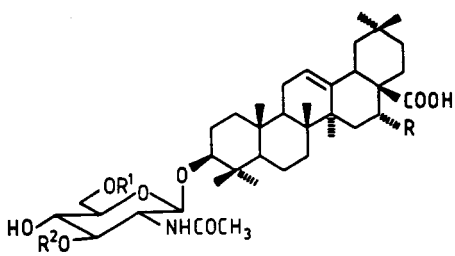
ABSTRACT.—The effects of aridanin [**1**] isolated from *Tetrapleura tetraptera* and of serotonin on the rhythmicity of the intestinal smooth muscle preparations of *Biomphalaria glabrata* were tested. Methysergide and cyproheptadine antagonized the contractile action of serotonin. Verapamil, lanthanum, and cyproheptadine inhibited the actions of aridanin, suggesting a calcium-dependent action of aridanin on gut tissue. Prolonged administration of aridanin significantly decreased the uptake of available calcium by the snails.

The molluscicidal property of *Tetrapleura tetraptera* Taub. (Mimosaceae), locally known as aridan, has been established (1–4). Aridanin [**1**] has been shown to reduce significantly the glycogen and protein content of *Biomphalaria glabrata*, indicating that this molluscicide may exert some of its effects on carbohydrate and protein metabolism (5). Serotonin is known to function as a neurotransmitter regulating molluscan visceral rhythmicity (6–8). In the gastrointestinal tract of the aplysiid gastropod mollusc *Apylysia dactylomela*, serotonin can induce rather complex effects when applied to the bathing medium over a range of concentrations (9). Ajimal and Ram (10) have shown that the action of serotonin can vary along the length of the gastrointestinal tract in *Apylysia*. We decided to investigate the effect of aridanin and serotonin on the gastrointestinal smooth muscle of *B. glabrata* with the hope that it might cast some light on the action of aridanin on the gut of this important schistosomal intermediate host.



1 R=R¹=R²=H

At low concentrations, serotonin (4.8×10^{-10} M) potentiated the amplitude of contraction in most of the esophageal (or intestinal) preparations examined. At median concentrations (1.92×10^{-8}–4×10^{-7} M), serotonin relaxed the tissue and increased phasic contractions in the relaxation phase of the esophagus in 30.0% of the preparations examined. High concentrations of serotonin (1.0×10^{-7}–9.6×10^{-6} M and 1.2×10^{-6}–1.9×10^{-5} M) produced contracture of the esophagus and intestine, respectively. Cyproheptadine (8.5×10^{-9}–3.2×10^{-8} M) and methysergide at the same range of concentrations antagonized this effect.

The effect of aridanin on intestinal preparations is very interesting. Aridanin (4×10^{-7}–3×10^{-6} M) enhanced the rhythmicity of the esophageal preparation as well as the intestine. In the intestine, contracture of the intestine was produced in most of the preparations examined. Contracture of the esophagus was accompanied by inhibition of the phasic contractions. Addition of a calcium antagonist, verapamil (2×10^{-7}

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M), lanthanum (10^{-4} M), or cyproheptadine (10^{-7} M), abolished both aridanin-induced contracture and potentiation of the rhythmicity of the intestine and esophagus of *B. glabrata*. This effect was not affected by methysergide (10^{-8}–10^{-7} M). Both aridanin-induced contraction and potentiation of the rhythmicity of the intestinal smooth muscles were antagonized by cyproheptadine (10^{-9}–10^{-7} M), lanthanum (10^{-4} M), and verapamil (10^{-7} M) but were resistant to atropine (10^{-6} M), methysergide (10^{-9}–10^{-7} M), and propanolol (10^{-5} M). On the other hand, serotonin-induced contraction was abolished by cyproheptadine (10^{-9}–10^{-7} M), lanthanum (10^{-4} M), and verapamil (2×10^{-7} M).

The effect of aridanin (0.125 and 0.25 ppm) on available calcium in *B. glabrata* test solutions is shown in Table 1. The table shows a time-dependent increase in the concentration of available calcium in *B. glabrata*-conditioned test solutions over a period of 10 weeks. This increase in the availability of calcium was found to be significant from the third week of continuous exposure to aridanin.

Serotonin at low concentrations potentiated the amplitude of contraction of the esophagus and the intestinal preparations of *B. glabrata*, while median concentrations (1.9×10^{-8}–4×10^{-7} M) decreased the muscular tone of the same tissues. Potentiation of the rhythmicity of the alimentary canal of *B. glabrata* at low concentrations resembles the serotonergic potentiation of the heart of *A. dactylomela* (16) and the buccal muscles (17) and rectum of *Apylysia* (10). The action of serotonin on the gut of *B. glabrata* suggests that this effect is not species specific.

Sublethal concentrations of aridanin were used in our studies on the effect of aridanin on the biology of *B. glabrata* (5,11). The enhancement of the rhythmicity of the gut of the snail was not mediated by endogenous substances like serotonin, acetylcholine, or catecholamines, as antagonists of these substances had no effect on the action produced by aridanin. This action was antagonized by specific calcium antagonists and cyproheptadine. Cyproheptadine (10^{-7} M) may be showing this effect because of its unspecific effect

TABLE 1. Ca^{++} Values in *Biomphalaria glabrata* Test Solutions.[a]

Week	Treatment	Calcium (ppm)
+1	0.25 ppm	28.3 ± 3.1
	0.125 ppm	28.2 ± 2.2
	Control	26.3 ± 4.2
p value		NS
+2	0.25 ppm	19.8 ± 5.2
	0.125 ppm	19.9 ± 7.2
	Control	18.7 ± 5.8
p value		NS
+3	0.25 ppm	24.2 ± 15.2
	0.125 ppm	22.2 ± 15.0
	Control	17.6 ± 5.2
p value		<0.05
+4	0.25 ppm	26.5 ± 6.4
	0.125 ppm	28.9 ± 5.5
	Control	25.1 ± 5.2
p value		<0.05
+5	0.25 ppm	19.0 ± 6.0
	0.125 ppm	18.7 ± 5.6
	Control	10.7 ± 7.2
p value		<0.05
+6	0.25 ppm	52.5 ± 14.2
	0.125 ppm	51.1 ± 11.4
	Control	28.9 ± 10.2
p value		<0.05
+7	0.25 ppm	73.0 ± 32.2
	0.125 ppm	68.7 ± 33.8
	Control	43.3 ± 15.2
p value		<0.05
+8	0.25 ppm	47.7 ± 8.0
	0.125 ppm	49.8 ± 8.4
	Control	28.6 ± 14.6
p value		<0.05
+9	0.25 ppm	70.8 ± 22.8
	0.125 ppm	69.6 ± 20.8
	Control	40.8 ± 16.0
p value		<0.05
+10	0.25 ppm	90.4 ± 2.0
	0.125 ppm	89.0 ± 8.1
	Control	55.6 ± 5.0
p value		<0.05

[a] $n = 24$, mean ± SD, NS = not significant. Continuously exposed to sublethal concentrations of aridanin (1.0 ppm aridanin = 1.5×10^{-6} M while 1.0 ppm $Ca^{++} = 2.5 \times 10^{-5}$ M).

at high concentrations (18). It is therefore more likely that the effect of aridanin on the muscle is calcium-dependent. This theory can be justified because prolonged administration of sublethal concentrations of aridanin (Table 1) over a period of 10 weeks consistently reduced the uptake of available calcium in test solutions when compared with untreated controls. The differences in ppm calcium for the control snails could be due to biological variation.

Saponin at a concentration of 50.0 μg/ml can be used for the chemical skinning of cardiac muscles (19). If skinning of any membrane is achieved, the cell will allow the entry of any ion or even a micromolecule (20); so the membrane potential can thus be completely abolished. The concentration of aridanin used in this study is low (4×10^{-7}–3×10^{-6} M), and the fact that aridanin exerts its effects even at these sublethal concentrations suggests that an aridanin-induced pertubation of membrane may be related to its molluscicidal activity (1–3), its effect on the growth and egg production of *B. glabrata* and *Lymnaea columella* (11), and its effect on carbohydrate and protein content of *B. glabrata* (5).

The present study indicates that a modification of the calcium channel could be involved in the action of aridanin. Because aridanin significantly affects carbohydrate and protein metabolism at almost the same concentrations, we suggest that there may be a metabolic alteration secondary to the effect of aridanin on the calcium channel. We intend to elucidate the mechanism(s) by which aridanin exerts its function in the control of the calcium channel and perform a detailed analysis of the action of serotonin in the gut of *B. glabrata*.

EXPERIMENTAL

SOURCE OF SNAILS AND TISSUE PREPARATION.—Specimens of *B. glabrata* (15–20) mm shell diameter) maintained at the Zoological Institute of the University of Hamburg were used. The snails were dissected as previously described (5) using a dissecting microscope. The esophagus or the intestine (that portion of the gut distal to the hepatopancreas extending to the rectum) was removed and placed in nutrient medium containing in mM per liter: NaCl, 47.9; KCl, 2.0; Na_2HPO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 1.8; $CaCl_2$, 3.6; $NaHCO_3$, 0.60.

The isolated esophagus or intestine was suspended under a tension of 100 mg in an aerated 50 ml bath at room temperature (20±2°). Movement was recorded by means of an isotonic transducer writing on a Siemens Kompensograph II (model M73923). Preparations were allowed to stabilize for 90–120 min, with regular changes of the bath solution every 20 min. In some experiments, the esophagus and intestine were used to test for changes in serotonin-induced contractions in the presence and absence of bath-applied methysergide, cyproheptadine, verapamil, lanthanum chloride, atropine, and propanolol. In other experiments, changes in aridanin-induced contractions in the absence and presence of methysergide, cyproheptadine, lanthanum chloride, and verapamil were investigated.

Two groups of snails were used as reported by Adewunmi *et al.* (11) to test the effect of sublethal concentrations (0.125 and 0.25 ppm) of aridanin on the uptake of calcium from test solutions containing snails over a period of 10 weeks. Twenty-four 4-week-old snails were placed in 800 ml of water in three polythene bags at each concentration of aridanin. Controls with no aridanin were included. The test solutions were changed every 3–4 days because aridanin has been found to be inactive in solution after standing for up to 5 days (11). Calcium concentration was determined using the EDTA-titration method of Golterman *et al.* (12). The results of the calcium concentration in test solutions were analyzed by analysis of variance using a computer program (13).

DRUGS.—Drugs used were: atropine sulfate (Sigma); methysergide hydrogen maleate (Sandoz); cyproheptadine (Merck, Sharp and Dohme); serotonin creatinine sulfate (Koch-Light); verapamil hydrochloride, lanthanum chloride (Knoll); propanolol (I.C.I.) and aridanin isolated from *T. tetraptera* (14,15).

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