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SHORT COMMUNICATIONS

Presence of endogenous digitalis-like activity in mammalian heart not due to fatty acids

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It has been observed that mammalian heart extracts contain endogenous material that inhibits $(Na^+ + K^+)$ATPase [1, 2], displaces [^{3}H]ouabain specific binding [1, 3], and cross-reacts with antibodies against cardiac glycosides [3–6]. However, its chemical nature remains to be elucidated [2, 6]. It is well known that free fatty acids are able to displace [^{3}H]ouabain specific binding [7]. Furthermore, arachidonic, oleic and related fatty acids have been reported to be the active material in tissue and plasma fractions that inhibit $(Na^+ + K^+)$ATPase and displace [^{3}H]ouabain binding [8, 9]. To examine if digitalis-like activity present in mammalian heart extracts is due to fatty acids, the abilities of ouabain, of arachidonic, oleic and linoleic acids, and of a partially purified extract from guinea pig heart to displace [^{3}H]ouabain specific binding to guinea pig heart $(Na^+ + K^+)$ATPase were assessed.

Methods

Digitalis-like factor preparation. Guinea pig hearts were extracted as previously described [4]. Briefly, the procedure comprised homogenization in deionized water and protein precipitation with methanol. After solvent evaporation, the dry matter corresponding to 57 g of original tissue was taken up in 20 ml of water. Aliquots (2 ml) were applied to 10 Sep Pak C-18 cartridges previously activated with methanol and water. After washing three times with 2 ml of water, the active fraction was eluted with 3 ml of methanol–water (80:20, v/v). The eluates were pooled and the solvent was evaporated. The dry residue was dissolved in 5 ml of 4 M pyridine-acetate, pH 7.0, and applied to a 20 × 4 cm column of Amberlite MB-3. Elution was carried out with 25 ml of the pyridine-acetate buffer. The eluent was then evaporated under low pressure. The preparation was ion-free as determined by atomic absorption. The extract concentration is expressed as the amount equivalent to 1 g of original tissue per ml (g tissue/ml).

[^{3}H]*Ouabain binding assay.* Guinea pig heart $(Na^+ + K^+)$ATPase was prepared according to De Pover and Godfraind [10]. A 5.5 μg sample of enzyme protein was incubated for 30 min at 37° in a medium containing 100 mM NaCl, 3 mM $MgCl_2$, 3 mM ATP, 1 mM EGTA, 25 nM [^{3}H]ouabain (18 Ci/mmol, New England Nuclear) and 20 mM maleate–Tris, pH 7.4. The final volume was 0.1 ml. The reaction was stopped by the addition of 1 ml of a chilled sucrose solution (0.25 M sucrose, 5 mM Tris–HCl, pH 7.4) and immediate filtration through Whatman GF/F filters. After washing with 20 ml of the sucrose solution, the filters were dissolved in 10 ml of toluene–Instagel (25:75, v/v) and the radioactivity of the samples was determined by liquid scintillation. Non-specific binding determined in the presence of 1 mM ouabain was less than 10%.

Statistics. Hill coefficients and standard errors (SE) were calculated by linear regression and compared by the test of parallelism described by Tallarida and Murray [11].

Results and discussion

[^{3}H]Ouabain specific binding displacement by unlabeled ouabain and by a partially purified extract from guinea pig heart is shown in Fig. 1. The K_d for the $(Na^+ + K^+)$ ATPase–ouabain complex calculated from the ouabain displacement curve by the Cheng–Prusoff equation [12], was 190 nM. This value is in agreement with those reported under similar conditions [10]. The EC_{50} for the extract was 25.1 g tissue/ml. Experimental data were very well fitted by simulated curves for purely competitive displacements. Hill coefficients were (±SE) 1.04 ± 0.02 for ouabain and 1.06 ± 0.09 for the extract, being not statistically significantly different from unity.

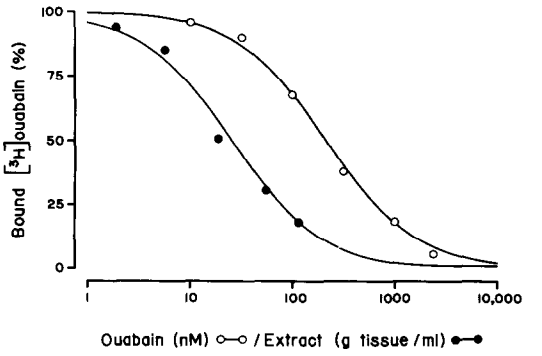

Fig. 1. Displacement of [^{3}H]ouabain specific binding to guinea pig heart $(Na^+ + K^+)$ATPase by unlabeled ouabain and by a partially purified extract from guinea pig hearts. [^{3}H]Ouabain binding displacement by the extract or ouabain was assayed on three different $(Na^+ + K^+)$ATPase preparations. Each point corresponds to the mean value; standard error of the mean did not exceed the diameter of the symbol. Traces are simulated curves for competitive displacements.

Arachidonic, oleic and linoleic acids also displaced [^{3}H]ouabain specific binding, but the displacement curves were steeper than that of unlabeled ouabain (Fig. 2). Similar results have been reported with fatty acid-enriched tissue and plasma fractions and with the chemically pure compounds [8, 9]. Hill coefficients were (±SE) 1.74 ± 0.15 for arachidonic acid, 2.71 ± 0.72 for oleic acid, and 2.52 ± 0.28 for linoleic acid. Values were statistically significantly different from unity, indicating that the displacements did not follow Michaelis–Menten kinetics. The steep displacement curves suggest that fatty acids produce a decrease in the number of ouabain binding sites. This is consistent with the proposition that free fatty acids inhibit the transition of $(Na^+ + K^+)$ATPase to the phosphorylated form [13], conformation that binds digitalis [14].

The results indicate that it is not likely that the activity present in the guinea pig heart extract was due to fatty acids since interaction with [^{3}H]ouabain at the binding site followed a different mechanism. The observation that the extract produced a competitive displacement of the specific radioligand binding suggests the presence of an endogenous ligand of the ouabain binding site. This study confirms previous reports [1–6] on the existence of endogenous digitalis-like activity in mammalian heart.

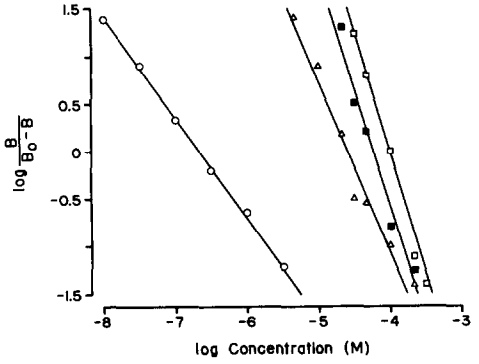

Fig. 2. Hill plots for displacements of [^{3}H]ouabain specific binding to guinea pig heart ($Na^+ + K^+$)ATPase by ouabain (○), arachidonic acid (△), oleic acid and (■) and linoleic acid (□). All displacing agents were assayed on three different ($Na^+ + K^+$)ATPase preparations; each point corresponds to the mean value. B_0 is the radioligand binding in the absence of displacing agent; B is the radioligand binding in the presence of displacing agent.

In summary, displacement of [^{3}H]ouabain specific binding to guinea pig heart ($Na^+ + K^+$)ATPase was produced by unlabeled ouabain, by a partially purified extract from guinea pig heart and by arachidonic, oleic and linoleic acids. Ouabain and the extract interacted with the radioligand at the binding site in a competitive manner, whereas fatty acids produced non-Michaelis displacements. Therefore, the extract activity is not likely due to the presence of fatty acids, but to an endogenous factor that binds to the digitalis binding site.

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Effects of opioid agonist drugs on the *in vitro* release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT from slices of rat globus pallidus

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The strio-pallidal enkephalin-containing pathway appears to influence locomotor activity and circling behaviour [1, 2]. But how enkephalins alter neuronal activity in the globus pallidus has not been investigated. Enkephalins may act to modulate afferent input to pallidum, or may directly alter the activity of output neurones.

The rat globus pallidus receives projections from many brain regions including the striatum [3, 4], substantia nigra [5], subthalmic nucleus [6, 7], nucleus accumbens [8, 9] and dorsal raphe nuclei [10, 11]. The strio-pallidal projection contains γ-aminobutyric acid (GABA) and this projection is involved in the mediation of circling behaviour [12]. Similarly, pallidal afferents from the nucleus accumbens also contain GABA but influence locomotor activity [13]. Collaterals extending from the nigro-striatal pathway may provide dopaminergic innervation to the globus pallidus [15]. While fibres projecting from the dorsal raphe nucleus give rise to 5-hydroxytryptamine (5HT) containing terminals [11].

In this work we have examined the action of opioid agonist drugs on the release of GABA, dopamine and 5HT in the globus pallidus. We have examined the effects of opiate agonists previously shown to induce circling or locomotor response on intrapallidal injection [1] to alter the release of ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT from pallidal slices.

Materials and methods

Tissue preparation and prelabelling of pallidal slices with ^{3}H-GABA, ^{3}H-dopamine and ^{3}H-5HT. Pallidal tissue from individual female Wistar rats (151–175 g; Charles River Ltd) was chopped in two directions (0.2 mm × 0.2 mm) using a McIlwain tissue chopper (Mickle Engineering Co. Ltd.). The resulting pallidal slices were dispersed in 1.0 ml of oxygenated Krebs buffer, pH 7.4, at 37°. Slices were prelabelled with ^{3}H-GABA (74 Ci/mmol; Amersham International), ^{3}H-dopamine (13.6 Ci/mmol. Amersham International) or ^{3}H-5HT (21 Ci/mmol; Amersham