Table 3. Effect of the concentration of pyrogallol on the oxidation *in vitro* of metallic mercury by horseradish peroxidase (HRP)*

HRP (26 μg)	Pyrogallol (mM)	H_2O_2 (%)	Mercury oxidation (μg/ml)	(μg/μmole protein)
+	16.7×10^{-1}	3	0.091 ± 0.012	155
+	16.7×10^{-2}	3	0.093 ± 0.009	157
+	16.7×10^{-3}	3	0.222 ± 0.027	376
+	16.7×10^{-4}	3	0.464 ± 0.032	784
+		3	0.541 ± 0.096	915

* The calculations of enzyme activity were based on a mol. wt of 44,000 for horseradish peroxidase; 26 μg corresponds to 6.0×10^{-4} μmole of horseradish peroxidase.

normal treated with aminotriazole, and acatalasemia treated with aminotriazole was 100:84:74:39 without hydrogen peroxide and 113:91:73:57 with hydrogen peroxide while the ratio of their catalase activities was 100:40:9:0.2.

The effects of ethanol and pyrogallol on mercury oxidation are under investigation.

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Interaction of lisuride with monoamine receptors on human blood platelets

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The ergot derivative lisuride, a simple semisynthetic 6-methyl-isoergolene derivative with a urea residue in position 8, has been introduced into clinical practice for treatment of migraine [1]. Moreover, it inhibits prolactin secretion and is used as an antiparkinsonian drug [2, 3]. Biochemical and pharmacological studies have shown that lisuride interacts with dopamine and serotonin receptors as well as with adrenoceptors [1, 3–7]. Direct binding studies indicate that ^{3}H-lisuride is bound specifically to α_2-adrenoceptors, dopamine$_2$- and high affinity serotonin receptors in CNS [8].

Since blood platelets are a particularly useful tool for studying aminergic reactions [9, 10], they were used to examine the influence of lisuride on monoamine receptors. Blood platelets possess receptors for catecholamines and serotonin which differ from the carrier for the active amine uptake [9, 11–13]. Interaction of catecholamines or serotonin with specific platelet receptors produces biochemical and morphological changes of platelets resulting in shape change and/or aggregation and release reaction [9, 12, 14]. We studied the influence of lisuride on the adrenaline-induced and serotonin- or dopamine-potentiated, ADP-induced aggregation. By comparison with the selective α_2-adrenoceptor blocking agent rauwolscine, the serotonin receptor antagonist pizotifen and the dopamine receptor antagonist haloperidol we attempted to demonstrate the specific effect of lisuride. Furthermore, we examined to what extent lisuride interferes with the ^{3}H-yohimbine binding in intact platelets.

Materials and Methods

The following substances were used: (−) Adrenaline bitartrate (VEB Jenapharm, GDR); serotonin: 5-hydroxytryptamine creatinine sulphate (Merck, F.R.G.); lisuride

hydrogen maleate (Schering, Berlin-West); rauwolscine hydrochloride (Roth, F.R.G.); yohimbine hydrochloride and dopamine hydrochloride (VEB Arzneimittelwerk Dresden, G.D.R.); pizotifen hydrochloride (Sandoz, Switzerland); haloperidol (Richter, Hungary); ADP (Reanal, Hungary); ^{3}H-yohimbine; yohimbine (methyl-^{3}H) (New England Nuclear, U.S.A.).

Blood was obtained by venipuncture from healthy volunteers who had not taken antiphlogistic agents for one week. The anticoagulant used was sodium citrate (9 vol. blood + 1 vol. 0.12 mM sodium citrate solution). Platelet-rich plasma (PRP) was prepared by differential centrifugation. For aggregation studies the PRP was adjusted to a platelet count of 2.5–3 × 10^8 platelets per ml with autologous platelet-poor plasma. Platelet aggregation was measured turbidimetrically at 37°. The PRP was preincubated with the inhibitor for 3 min before inducing aggregation.

The aggregating agents used (in final concentrations) were adrenaline (5 μM), serotonin (1 μM), dopamine (30 μM) and ADP (0.5–2 μM). To potentiate aggregation, an ADP concentration was chosen producing approx. 10–20% of the maximum effect. Simultaneous addition of serotonin (1 μM) increased ADP-induced aggregation 2–3 times. The dopamine concentration used (30 μM) did not affect adrenaline-induced aggregation, whereas it was inhibited at concentrations beyond 60 μM.

Percent inhibition of aggregation was calculated by comparing the maximal decrease in optical density of the control with the maximal decrease in optical density of the experimental samples. In the calculation of the synergistic serotonin effect the effect of ADP was ignored. Each compound was tested in four different plasmas at least.

To assess the serotonin uptake, the PRP was incubated with ^{14}C-serotonin (specific activity 58 mCi/mmol) at concentrations of 0.25 and 2.5 μM for 15 min. The radioactivity in the platelets was measured according to [15].

For binding assays with ^{3}H-yohimbine the platelets were washed twice with buffer (50 mM Tris, 100 mM NaCl, 5 mM EDTA, pH 7.5) and resuspended in buffer containing 2 × 10^9 platelets per ml.

For binding assays, 100 μl aliquots of the platelet suspension were incubated with 50 μl buffer or solution of the test substance or of unlabelled yohimbine (final concentration 50 μM), respectively, at 25° for 20 min, then 50 μl ^{3}H-yohimbine (2.5 nM) were added and incubated at 25° for 20 min. The incubation was terminated by diluting the incubation mixture with 2 ml buffer followed by rapid vacuum filtration through a Whatman glass fibre filter GF/C. The assay tube and the filter were rapidly washed with 30 ml buffer. The radioactivity was measured in an automatic liquid scintillation counter (LKB Wallac 81,000). Specific binding was defined as the difference between total binding and nonspecific binding in the presence of unlabelled yohimbine.

Results

Inhibition of serotonin-induced platelet aggregation. In contrast to serotonin, dopamine or adrenaline, lisuride up to a concentration of 100 μM did not cause platelet activation and had no synergistic effect, for instance in ADP-induced aggregation. Lisuride at a concentration of 1.0–10.0 μM did not inhibit the ^{14}C-serotonin uptake by blood platelets.

In platelet-rich human citrated plasma serotonin caused slight reversible aggregation. To examine the influence of lisuride on the serotonin action, we therefore utilized the synergistic effect of serotonin on ADP-induced aggregation. Lisuride did not affect ADP-induced aggregation even if high concentrations were used (>0.1 mM). However, it antagonised the synergistic serotonin effect on the ADP-induced response. The inhibitory effect occurred from a concentration of 1 nM and reached a maximum at 1 μM, as shown in the concentration–response curves (Fig. 1). Lisuride was nearly equieffective to the serotonin antagonist pizotifen. Compared to lisuride, haloperidol and rauwolscine were 2–3 orders of magnitude less effective.

Inhibition of adrenaline- and dopamine-induced platelet aggregation. In previous studies [12] lisuride has been found to be a potent inhibitor of adrenaline-induced aggregation. It was equipotent to rauwolscine as shown by the concentration–response curves for inhibition of aggregation (Fig. 2(B)). The effect of lisuride occurred rapidly; prolongation of incubation time in PRP did not enhance the inhibitory effect. Pizotifen and haloperidol were able to inhibit adrenaline-induced aggregation; however, they were more than two orders of magnitude less effective than lisuride.

Dopamine alone at a concentration of 0.1–100.0 μM did not induce aggregation in human PRP. However, it potentiated aggregation induced by low ADP concentrations. An experiment is shown in Fig. 3. The synergistic effect of dopamine was nearly completely blocked by lisuride and rauwolscine at a concentration of 0.3 μM. The inhibitory effect of haloperidol required a concentration of 60.0 μM.

Displacement of ^{3}H-yohimbine binding to intact platelets. ^{3}H-Yohimbine proved to be a suitable radioligand for α_2-adrenoceptors on intact blood platelets [16]. The binding was rapid, saturable and reversible. From the Scatchard plot a K_d of 1.92 nM was determined [16]. Lisuride and

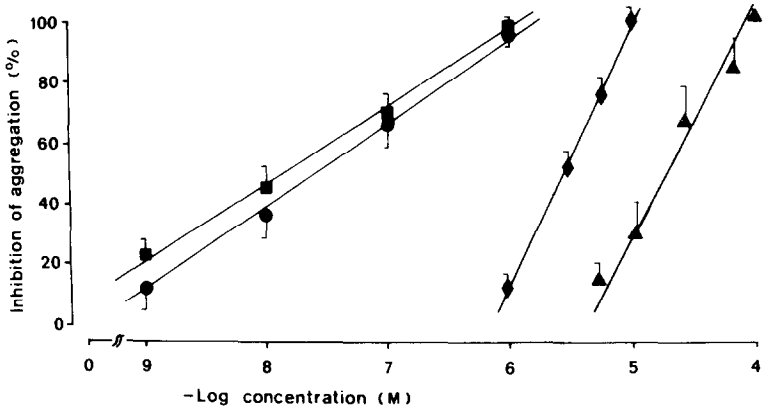

Fig. 1. Concentration–response relationships for inhibition of serotonin-potentiated, ADP-induced aggregation by lisuride (●), pizotifen (■), haloperidol (◆) and rauwolscine (▲). Means ± S.E.M., n = 4–6.

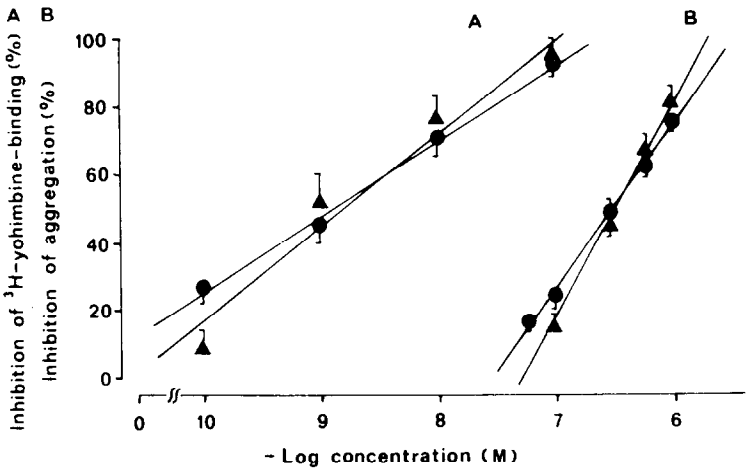

Fig. 2. Concentration–response relationships for inhibition of ^{3}H-yohimbine (2.5 nM) binding to intact human platelets (A) and for inhibition of adrenaline-induced aggregation (B) by lisuride (●) and rauwolscine (▲). Means ± S.E.M., $n = 6$.

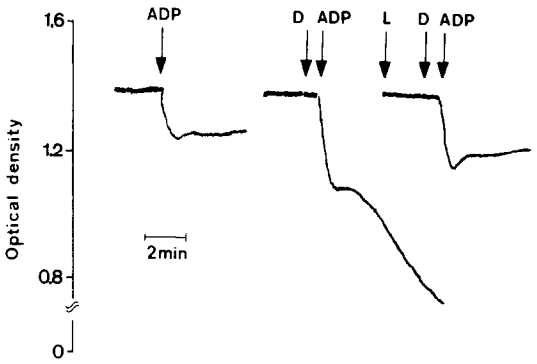

Fig. 3. Dopamine (D: 30 μM)-potentiated, ADP (2 μM)-induced aggregation and inhibition by lisuride (L: 0.3 μM).

rauwolscine inhibited effectively the binding of ^{3}H-yohimbine to human platelets (Fig. 2(A)). Pizotifen at a concentration of 1.0 μM reduced ^{3}H-yohimbine binding by about 20%.

Discussion

In our studies on the lisuride-receptor interaction we used platelets since they are a receptor-bearing tissue easily obtainable from man. Lisuride had no agonist activity on blood platelets; however, it proved to be a potent inhibitor of the aminergic platelet reaction. Lisuride has been found to act at peripheral serotonin receptors as an antagonist [1, 17]. This was also demonstrated in serotonin receptors on platelets. At very low concentrations it inhibited the synergistic serotonin effect on ADP-induced aggregation and was approximately equipotent to the specific serotonin receptor antagonist pizotifen. In rabbit platelets it inhibited also serotonin-induced shape change to the same extent as methysergide and (+)LSD [14].

Studies with selective radioligands in frontal cortex membranes from animals have shown that lisuride interacts both with serotonin$_2$ and serotonin$_1$ receptors [7, 8]. According to the relative potencies of several serotonin receptor antagonists, the serotonin receptors of platelets can be assigned to the functional serotonin$_2$ receptors occurring in the frontal cortex and in vascular smooth muscle [18–21]. The presence of serotonin$_2$ receptor binding sites on cat platelets has been demonstrated using [^{3}H] ketanserin [22]. Therefore, lisuride is believed to belong to the antagonists with high affinity for this receptor subtype.

Battaglia and Titeler have found that ^{3}H-lisuride is bound specifically to α_2-adrenoceptors of bovine frontal cortex membranes [8]. Our results corroborated this finding. We found that lisuride inhibited adrenaline-induced aggregation mediated by α_2-adrenoceptors and the specific binding of ^{3}H-yohimbine to intact platelets at nanomolar concentrations. Rauwolscine which blocks preferentially α_2-adrenoceptors possessed the same inhibitor strength as lisuride. Thus, lisuride belongs to the ergoline derivatives with high affinity for α_2-adrenoceptors such as dihydroergotamine. In this context it is noteworthy that bromocriptine is a considerably weaker antagonist than lisuride [12].

Since lisuride acts preferentially as a central dopamine receptor agonist [4, 5, 7] its effect on the dopamine-induced aggregation was of interest. For serotonin and adrenaline specific binding sites on platelets have been demonstrated [13, 23]; however, there is no evidence for the existence of a specific dopamine receptor or a transport carrier analogous to the dopaminergic neurons [24]. The synergistic effect of dopamine on ADP-induced aggregation appears to be mediated through α_2-adrenoceptors because lisuride and rauwolscine antagonise this effect in the same concentration range. The inhibitory effect of the dopamine receptor antagonist haloperidol requires comparatively high concentrations such as those used to suppress adrenaline-induced aggregation.

In conclusion, lisuride proved to be a powerful antagonist of α-adrenoceptor and serotonin receptor subtypes on platelets. It was equipotent to other specific antagonists of these receptors. The antiadrenergic and antiserotonergic effects of lisuride might be of some consequence to adrenaline- and serotonin-potentiated aggregation *in vivo*. The present studies indicate that ergot derivatives, at least lisuride, display a variety of pharmacological actions since they are capable of interacting with various specific receptors.

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A methodical pitfall: saturable binding of digitoxin to glass-fibre filters

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In vitro radioligand binding studies have become a standard procedure in experimental pharmacology for the investigation of drug–receptor interactions. Concerning the interpretation of experimental results revealing saturable binding of a radioligand to biological membranes, reservation is recommended, since saturable binding does not necessarily mean binding to pharmacologically relevant receptors [1]. Furthermore, it has been shown that radioligands even may bind to non-biologic materials, e.g. Cuatrecasas and Hollenberg [2] demonstrated a high affinity binding of [^{125}J]insulin to talc powder and Synder *et al.* [3] communicated on a stereoselective binding of opiates to glass-fibre filters.

In investigations on the characteristics of the interaction of cardiac glycosides with their receptor, i.e. the sarcolemmal Na^+/K^+-ATPase (ATP phosphohydrolase, E.C. 3.6.1.3), binding studies using radioactively labelled cardiac glycosides are a tool often applied [4]. Also in our laboratory the measurement of [^{3}H]ouabain-binding to cardiac membranes is a well established method [5, 6]. However, when employing our routine procedure to the measurement of [^{3}H]digitoxin-binding, we were initially troubled by the finding that the characteristics of "specific" digitoxin-binding differed essentially from that of ouabain-binding. Further analysis revealed the reason, namely a saturable, medium-affinity binding of digitoxin to glass-fibre filters.

We consider it worthwhile to communicate this finding in order to demonstrate that the above-mentioned precautions are also valid in experiments on the specific binding of cardiac glycosides, since otherwise misleading interpretations of experimental data and of Scatchard analyses may result.

Materials and Methods

[^{3}H]Digitoxin (13.8 Ci/mmol) and [^{3}H]ouabain (32 Ci/mmol) were obtained from NEN (Dreieich, F.R.G.) and Amersham Buchler (Braunschweig, F.R.G.), respectively. Digitoxin (puriss.) and Digoxin (purum) were purchased from Fluka (Neu-Ulm, F.R.G.). Ouabain and the other chemicals were obtained from E. Merck (Darmstadt, F.R.G.) unless otherwise indicated.

The procedure of the binding experiments has been described earlier in detail [5, 6]. A crude suspension of cardiac membranes from guinea pig cardiac ventricles was prepared at a temperature of 4° as follows. The ventricles were homogenized in a 0.32 M sucrose solution (20 ml/g wet weight); the homogenate was centrifuged at 2000 *g* for 10 min; the supernatant was centrifuged at 30,000 *g* for 18 min; the pellet was resuspended in 50 mM Tris–HCl buffer pH 7.4 (4 ml/g w.w.), frozen in liquid nitrogen and stored at −20°.

The binding assays were performed in triplicate in glass vials (filtration experiments) or in thick-wall polyallomer centrifugation tubes (Beckman, Hannover, F.R.G.). In a