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Effect of ouabain, digoxin and digitoxigenin on potassium uptake and histamine release from rat peritoneal mast cells

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Rat peritoneal mast cells were used to study the effects of digitalis glycosides on potassium uptake and histamine release induced by compound 48/80, substance P and egg-albumin (immunological release). In the absence of calcium all glycosides inhibited potassium uptake. Ouabain and digoxin enhanced the histamine release while digitoxigenin either had no effect or was slightly inhibitory. In the presence of calcium, the glycosides only affected potassium uptake and histamine release slightly. In the presence of lithium or lanthanum the enhancement of the histamine release was counteracted. Hydrophilic digitalis glycosides seem to enhance histamine release secondary to an increase in intracellular sodium. Lipophilic glycosides have no effect on the release.

Mast cell; Histamine; Secretion; Exocytosis; Digitalis glycoside; Na^+/K^+-pump; Na^+/K^+-ATPase

1. INTRODUCTION

The digitalis glucoside ouabain, enhances histamine release from mast cells incubated in a calcium-free medium [1–4]. The Na^+/K^+-pump in the plasma membrane of mast cells [5] is inhibited by ouabain, and this allows the accumulation of sodium inside the cells. The enhancement by ouabain of histamine release develops gradually over 0.4–1 h [2,3], and it is likely to be related to the accumulation of sodium, which may induce changes in a pool of calcium bound to the plasma membrane [4]. Erjavec and Ferjan [6] reported that hydrophilic digitalis glycosides enhance histamine release from rat mast cells induced by compound 48/80, while more lipophilic glycosides inhibit histamine release. Measurements of the activity of the Na^+/K^+-pump was, however, not performed in that study.

The aim of this investigation was to compare the effect of three digitalis glycosides (ouabain, digoxin and digitoxigenin) on the release of histamine from rat mast cells with the effect of the glycosides on Na^+/K^+-pump activity (the ouabain-sensitive potassium uptake). While ouabain is hydrophilic and digitoxigenin is lipophilic, digoxin is in between [7]. Additionally it was studied whether the mechanism behind ouabain enhancement of compound 48/80-induced histamine release also applied to release induced by substance P or egg-albumin (immunological release).

Measurements of cellular potassium uptake was performed by use of $^{86}Rb^+$ as a tracer for potassium (K^+($^{86}Rb^+$)-uptake).

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2. MATERIALS AND METHODS

2.1. *Isolation of mast cells*

Male Sprague–Dawley rats, 200–400 g, were used for the experiments with compound 48/80 and substance P, and male Wistar rats, 340–440 g, were used for the experiments with egg-albumin. The rats were killed by decapitation under light ether anaesthesia. 10 ml of Krebs–Ringer solution was prewarmed to 37°C and injected into the abdominal cavity through a small incision in the abdominal wall. After massage for 1 min, the Krebs-Ringer solution containing mixed peritoneal cells was aspirated. The peritoneal mast cells were isolated on a self-generating gradient of Percoll as previously described [5]. The cells were suspended in an appropriate volume of Krebs–Ringer solution for the experiments.

The number of cells was measured by an automatic cell counter, and the purity of the suspensions was determined by inspection of stained (Toluidine blue) smears. The fraction of mast cells was 98.6 ± 1.2% (mean ± S.D., $n = 40$).

2.2. *Sensitization of the rats*

Male Wistar rats were sensitized to egg-albumin by injecting 1 ml of pertussis vaccine with 25 mg of egg-albumin subcutaneously on the first day, followed by booster doses of 25 mg on the second and the third day. On the second day, the booster dose was given together with 0.5 ml of Freund's incomplete adjuvant. The rats were used 3–6 weeks after the first injection.

2.3. *Incubation procedure*

Mast cell suspensions pooled from 1–7 rats were divided into samples containing equal numbers of cells and preincubated in a final volume of 400 μl at 37°C. The details regarding time of preincubation and the presence of various chemicals and drugs are given in the legends to the figures. All calculations are based on duplicate samples. When histamine release was induced immunologically (egg albumin), the preincubation was performed in the presence of phosphatidylserine, 0.05 mg · ml^{-1}, which is known to enhance immunologically induced histamine release [8]. Each sample contained between

5.8×10^4 and 3.7×10^5 cells. Following the preincubation period, 100 μl of a Krebs–Ringer solution containing either trace amounts of $^{86}Rb^+$ (for the determination of the $K^+(^{86}Rb^+)$-uptake) or histamine secretagogues (for determination of the histamine release) were added, and the cells were incubated for 10 min. The incubation was terminated by addition of 9.5 ml ($K^+(^{86}Rb^+)$-uptake) or 1.75 ml (histamine release) of ice-chilled Krebs–Ringer solution.

2.4. *Measurement of the $^{86}Rb^+$-uptake of mast cells*

The concentration of $^{86}Rb^+$ during the incubation of the cells was between 1 and 150 μM, and the specific activity was 11.7 MBq per mmol of K^+, while the radioactive concentration was kept at 1.5 μCi ml^{-1}. The incubation was terminated and the cellular potassium uptake calculated as described elsewhere [4]. In Fig. 3, the ouabain-sensitive $K^+(^{86}Rb^+)$-uptake is shown. This is equivalent to the difference in uptake between cells incubated in the absence and presence of 1 mM ouabain.

2.5. *Measurement of cellular histamine release*

The concentrations of the histamine releasers during the incubation of the cells were as follows: compound 48/80, 1.0 $\mu g \cdot ml^{-1}$; substance P, 30 μM, and egg-albumin, 0.5 $mg \cdot ml^{-1}$. After termination of the incubation, the histamine release was determined fluorimetrically as previously described [4]. The histamine release was expressed as a percentage of the total histamine content of the cells and presented after subtraction of the spontaneous histamine release from cells incubated in the absence of the histamine releasers. The spontaneous histamine release was always less than 8.3%, except for the experiments in which extracellular sodium was substituted with lithium (Fig. 3). The spontaneous histamine release in the presence of lithium was up to 16.8% (range 14.2–16.8%). The digitalis glycosides and lanthanum had no significant effect on the spontaneous histamine release.

2.6. *Solutions*

The calcium-free Krebs–Ringer solution had the following composition (in mM): NaCl 136, KCl 4.75, $MgSO_4$ 1.2, Tris-HCl 12.5. The calcium-containing Krebs-Ringer solution contained (in mM): NaCl 134, KCl 4.75, $CaCl_2$ 1.0, $MgSO_4$ 1.2, Tris-HCl 12.5. In some of the experiments shown in Fig. 3, NaCl was substituted with an equimolar concentration of LiCl. All solutions contained bovine serum albumin, 1 $mg \cdot ml^{-1}$, and glucose, 1 $mg \cdot ml^{-1}$. The pH was 7.4 (room temperature).

2.7. *Materials*

Bovine serum albumin, digoxin, digitoxigenin, compound 48/80, substance P and phosphatidylserine was supplied by Sigma Chemical Co. (St. Louis, USA), glucose and dimethylsulphoxide (DMSO) by Merck (Darmstadt, Germany), Percoll by Pharmacia Fine Chemicals (Sweden), scintillation liquid (Ecoscint) by BN Plastics (Helsinge, Denmark), $^{86}Rb^+$ (specific activity 1–12 MCi/mg Rb^+) by Amersham (Buckinghamshire, UK), ouabain by Mecobenzon (Denmark), egg-albumin by Mallicrodt Chemical Work (St. Louis, USA). Freunds incomplete adjuvant and pertussis vaccine was supplied by Statens Serum Institut (Denmark). All other chemicals were of analytical grade. Ouabain was prepared as a 5 mM stock solution by dissolution in Krebs–Ringer solution. Digoxin and digitoxigenin were prepared as a 30 mM stock solution by dissolution in DMSO and diluted with Krebs–Ringer solution for the experiments. The concentration of DMSO during the experiments was maximally 1.7% (v/v). When digoxin and digitoxigenin were used, the control samples contained the same concentrations of DMSO but were without the glycosides. Compound 48/80 was prepared as a 1 $mg \cdot ml^{-1}$ stock solution by dissolution in 0.9% saline. Phosphatidylserine was prepared as a 0.5 $mg \cdot ml^{-1}$ stock solution by dissolution in Krebs–Ringer solution. Substance P was preared as a 500 μM stock solution by dissolution in Krebs–Ringer solution.

2.8. *Presentation of results*

All results are shown as mean ± S.E.M. of five experiments. The Mann–Whitney *U*-test was used for the statistical analysis of the data. $P < 0.05$ was chosen as the threshold level of significance.

3. RESULTS

The effect of ouabain, digoxin and digitoxigenin on cellular $K^+(^{86}Rb^+)$-uptake is shown in Fig. 1. While 10^{-5} M of the three drugs had no effect on the uptake, it was decreased in the presence of 10^{-4} M of the drugs. A maximal decrease to 20 ± 2% of the control value was observed with 10^{-3} M ouabain. Concentrations of the glycosides less than 10^{-5} M tended to increase the cellular uptake of potassium. The control values were (in pmol/10^6 cells/min): 463 ± 76 (ouabain), 461 ± 48 (digoxin) and 695 ± 130 (digitoxigenin).

Fig. 2 shows the effect of ouabain, digoxin and digitoxigenin on cellular $K^+(^{86}Rb^+)$-uptake and release of histamine induced by compound 48/80 (a), egg-albumin (b) and substance P (c). The same pattern of responses to the drug treatment were found with the three secretagogues. In a calcium-free medium the cellular uptake of $K^+(^{86}Rb^+)$ was inhibited by 10^{-4} M of digoxin, digitoxigenin and ouabain, as well as of 10^{-3} M ouabain. Except for digitoxigenin, which had no effect on histamine release, these drugs also enhanced the release of histamine. These effects were statistically significant except for the values (in Fig. 2a) of cellular $K^+(^{86}Rb^+)$-uptake in presence of 10^{-4} M of digoxin, digitoxigenin and ouabain. In the presence of calcium the drugs had no effect on the cellular $K^+(^{86}Rb^+)$-uptake. However, a slight increase, that reached the level of significance, was observed in the presence of 10^{-4} M digoxin and 10^{-3} M ouabain (Fig. 2c). In general, the drugs had only minor and mostly insignificant effects on the release of histamine in a calcium-containing medium. It is demon-

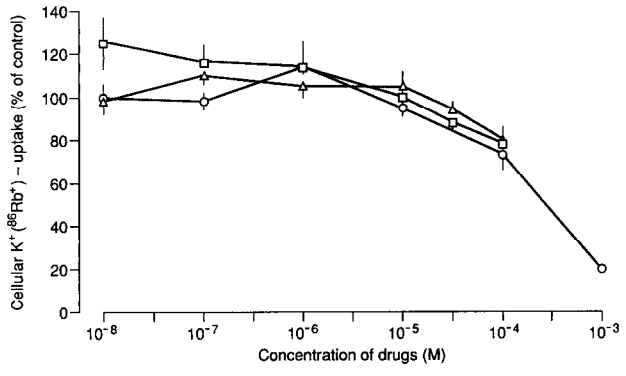

Fig. 1. Effect of ouabain, digoxin and digitoxigenin on the cellular $K^+(^{86}Rb^+)$-uptake. The cells were preincubated for 60 min at 37°C in a calcium-free medium containing 4.75 mM potassium and ouabain (○), digoxin (□) or digitoxigenin (△) in concentrations as indicated on the abscissa scale. Control samples without the glycosides were run in parallel. After preincubation 100 μl of Krebs–Ringer solution containing $^{86}Rb^+$ was added and the cells were incubated for 10 min. The ordinate scale shows the $K^+(^{86}Rb^+)$-uptake as a percentage of the control values.

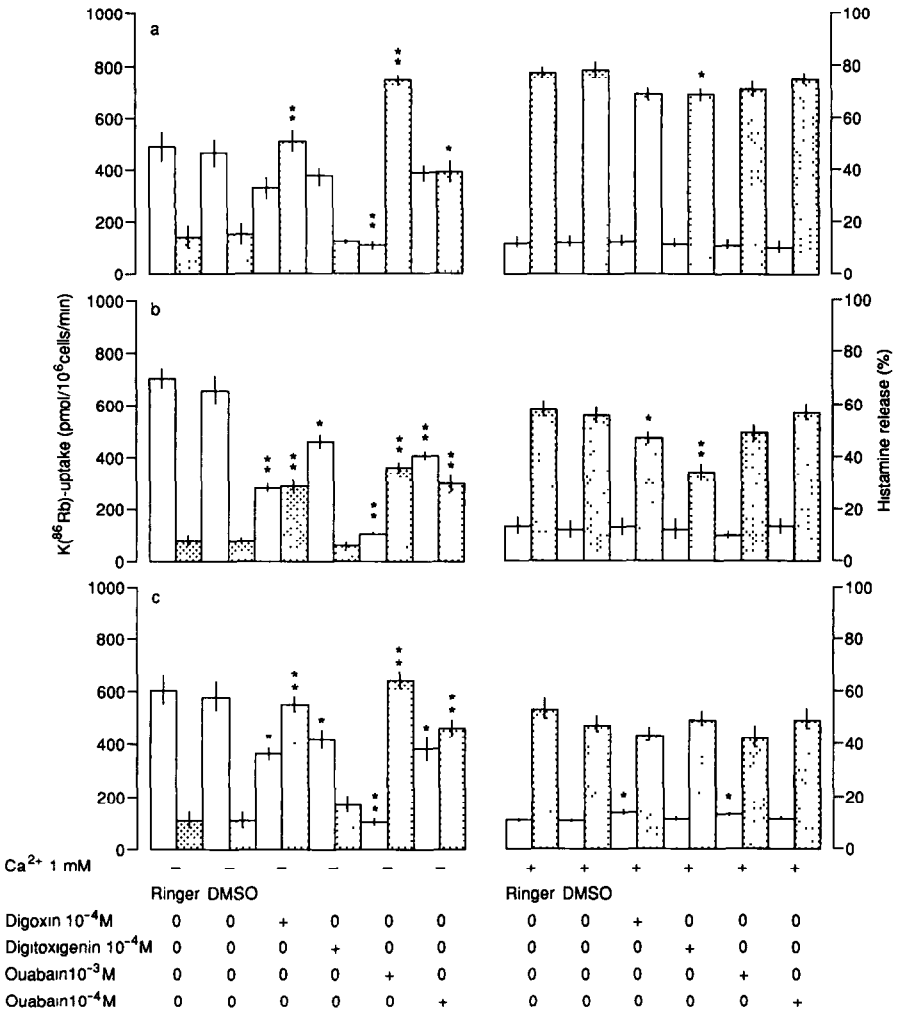

Fig. 2. Effect of ouabain, digoxin and digitoxigenin on cellular $K^+(^{86}Rb^+)$-uptake and histamine release induced by compound 48/80 (a), egg albumin (b) and substance P (c). The cells were preincubated for 60 min at 37°C in the absence or presence of 1 mM calcium in a medium containing 4.75 mM potassium and in the presence of the glycosides as indicated. Control samples without the glycosides were run in parallel. Following preincubation 100 μl of Krebs–Ringer solution containing either $^{86}Rb^+$ (for determination of $K^+(^{86}Rb^+)$-uptake) or compound 48/80 (a), egg-albumin (b), substance P (c) (for determination of cellular histamine release) was added and the cells were incubated for 10 min. For determination of spontaneous histamine release, samples without the secretagogues were run in parallel. The left hand scale shows the $K^+(^{86}Rb^+)$-uptake of the cells in pmol/10^6 cells/min (open columns), while the right hand scale shows the histamine release as a percentage of the total histamine content of the sample (hatched columns). $*P < 0.05$; $**P < 0.01$.

strated in Fig. 2a–c that, compared with control values from cells incubated without any drug or DMSO, the inclusion of DMSO (1.7% v/v) did not change the values for $K^+(^{86}Rb^+)$-uptake or histamine release induced by the three secretagogues either in the presence or in the absence of extracellular calcium.

Fig. 3 shows the effect of $LaCl_3$ and the replacement of extracellular sodium for lithium on the ouabain-sensitive $K^+(^{86}Rb^+)$-uptake and cellular histamine release induced by compound 48/80, egg-albumin and substance P. There was a large inhibition of the ouabain-sensitive $K^+(^{86}Rb^+)$-uptake by incubation of the cells with 1 μM $LaCl_3$ or replacement of sodium for lithium in the three groups of experiments.

Histamine release induced by the three secretagogues was increased significantly by addition of 10^{-3} M ouabain. Ouabain had no effect on the release of histamine when sodium was replaced with lithium. Addition of ouabain to $LaCl_3$-treated cells caused a small but significant increased histamine release with compound 48/80 and egg-albumin. In the presence of $LaCl_3$, the histamine release induced by substance P was not changed significantly by addition of ouabain.

4. DISCUSSION

The moderate decrease in $K^+(^{86}Rb^+)$-uptake of mast cells by 10^{-4} M of the lipophilic as well as the hydrophilic glycosides (Figs. 1 and 2) is likely to occur through inhibition of the activity of the Na^+/K^+-pump

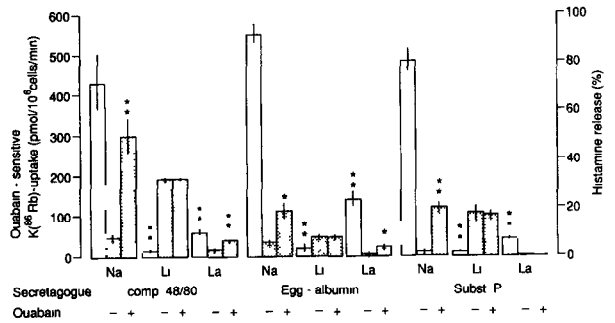

Fig. 3. Effect of ouabain, Li^+, and La^{3+} on cellular $K^+(^{86}Rb^+)$-uptake and histamine release induced by compound 48/80, egg-albumin and substance P. The cells were preincubated for 60 min at 37°C in presence of Li^+ or La^{3+} as indicated. Control samples preincubated in normal sodium-containing medium were included. In all cases the concentration of potassium was 4.75 mM. Following the first preincubation period, 100 μl of Krebs–Ringer solution containing ouabain (final concentration 10^{-3} M) was added to the cells for an additional preincubation period of 60 min. Samples without ouabain were included. The second preincubation was followed by an incubation period of 10 min in presence of ^{86}Rb (for determination of the $K^+(^{86}Rb^+)$-uptake) or compound 48/80, egg-albumin, substance P (for determination of histamine release). For the determination of spontaneous histamine release, samples without the releasers were run in parallel. The left hand scale shows the ouabain-sensitive $K^+(^{86}Rb^+)$-uptake (open columns) and the right hand scale represents the histamine release as a percentage of the total histamine content of the sample (hatched columns). $*P < 0.05$; $**P < 0.01$.

[5] since this is the cellular receptor for all cardiac glycosides [9].

The high concentration of ouabain (10^{-3} M) necessary to obtain a marked inhibition of the pump activity (Fig. 1) can be explained on a genetic basis [10], and this observation confirms previous reports on isolated rat Na^+/K^+-ATPase [11,12] and studies on cellular potassium uptake mediated by the Na^+/K^+-pump in rat liver cells [13]. The tendency of low concentrations of digitalis glycosides to increase the uptake of $K^+(^{86}Rb^+)$ has been reported previously in cardiac tissue [14].

DMSO was used to dissolve digoxin and digitoxigenin, and DMSO is known to inhibit the histamine release induced by compound 48/80 from rat peritoneal mast cells [15]. Because of this, the concentrations of digoxin and digitoxigenin was not increased above 10^{-4} M. The control experiments showed that the final concentration of DMSO of 1.7% (v/v) relevant at this concentration of the glycosides influenced neither the histamine release nor the Na^+/K^+-pump activity.

The previous findings that inhibition by ouabain of the pump activity in a calcium-free medium was associated with an enhancement of the release of histamine [1,2,4] was also observed in this investigation with digoxin but not with digitoxigenin. We have proposed that inhibition of the Na^+/K^+-pump by ouabain enhanced the compound 48/80-induced histamine release through an increase in the intracellular sodium concentration [4]. The effect of lithium (Fig. 3) indicates that this mechanism is also relevant for the release of histamine induced by egg-albumin and substance P. The dissociation between inhibition of the Na^+/K^+-pump activity by digitoxigenin and the lack of enhancement of histamine release may be explained by an additional effect of the drug on mast cells. The lipophilic character of the drug makes it likely that digitoxigenin could penetrate the plasma membrane and influence the transmembranal signal transduction or the fusion process of exocytosis.

The enhancement of histamine release by ouabain in a sodium-containing medium was almost blocked by lanthanum. This effect may be attributed to displacement by lanthanum of membrane-bound calcium [16], which is considered to be involved in stimulus–secretion coupling [17].

Our data indicate a role of membrane-bound calcium in the mechanism by which ouabain enhances the release of histamine by compound 48/80, substance P and egg-albumin from sensitized mast cells. We have suggested that ouabain increases the amount of membrane-bound calcium. This could occur as a consequence of an increased concentration of intracellular sodium. It has also been demonstrated that digitalis glycosides are able to increase the amount of calcium bound to plasma membranes by a mechanism independent of the inhibitory effect on the Na^+/K^+-pump [18]. The enhancement by ouabain thus seems to involve both intracellular sodium and membranal calcium in the exocytotic process, even when different secretagogues are used.

The low level of $K^+(^{86}Rb^+)$-uptake in the presence of calcium confirm previous observations and may be explained by an inhibitory effect of calcium on the cellular sodium uptake with a subsequent inhibition of the Na^+/K^+-pump [5,19]. This mechanism may also concern the inhibition observed with lanthanum [20]. In the presence of calcium in the medium ouabain had only a marginal effect on $K^+(^{86}Rb^+)$-uptake [5], as also observed in this study with digoxin and digitoxigenin.

In conclusion, in a calcium-free medium, the digitalis glycosides, ouabain and digoxin, increase the secretion of histamine from rat peritoneal mast cells induced immunologically and by compound 48/80 or substance P. In contrast, the more lipophilic glycoside, digitoxigenin, either has no or a small inhibitory effect on histamine release. The various glycosides do not differ in their inhibitory effects on the Na^+/K^+-pump. The difference between the various glycosides in their effects on the cellular release of histamine cannot be explained in terms of different effects on the Na^+/K^+-pump, but it may be due to an interaction between the lipophilic digitoxigenin and the plasma membrane. The mechanisms responsible for the enhancing effect of ouabain on the cellular histamine release seem to involve an increase in intracellular sodium and changes in a membrane-bound pool of calcium. These mechanisms are the same whether histamine release is induced immunol-

ogically (egg-albumin), by compound 48/80 or by substance P.

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