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Effects of ouabain on isolated cerebral and femoral arteries of the cat: a functional and biochemical study

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1 This study analyzes the mechanisms involved in the responses to ouabain in cat cerebral and femoral arteries and characterizes the electrogenic Na^+ pump present in these vessels. The latter was accomplished by measurement of [^{3}H]-ouabain binding to arterial membrane fractions, K^+-elicited relaxation and ouabain-sensitive $^{86}Rb^+$ uptake.

2 Ouabain induced transient contraction in cylindrical segments of cerebral arteries. This contraction was reduced by verapamil (3×10^{-6} M) and Ca^{2+}-removal from the medium but was not modified by phentolamine (3×10^{-6} M) or pretreatment with reserpine. However, the contraction elicited by ouabain in femoral artery segments lasted longer, and was reduced by Ca^{2+}-omission, phentolamine or reserpine, but remained unaffected by verapamil.

3 The immersion of the arteries in low-Na^+ (25 mM) medium abolished the contraction caused by ouabain.

4 The exposure of the arteries to a K^+-free medium induced a small transient increase in tension, and the subsequent application of K^+ (7.5 mM) elicited a marked relaxation. This effect was greater in cerebral than in peripheral arteries, and was suppressed by ouabain (10^{-4} M).

5 Scatchard analysis of the [^{3}H]-ouabain binding to arterial membrane fractions suggested a single class of binding sites. The K_D values for both kinds of arteries were of similar order, while the B_{max} value was greater in cerebral than in femoral arteries.

6 Total and ouabain-sensitive $^{86}Rb^+$ uptakes were greater in cerebral than in femoral vessels.

7 These results indicate that: (1) ouabain-induced contraction of cerebral arteries is due to a direct effect on vascular smooth muscle cells, while in femoral arteries it is due to noradrenaline release from adrenergic nerve terminals; and (2) the electrogenic Na^+ pump activity is greater in cerebral than in peripheral arteries.

Introduction

Sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase) is considered to play an important role in the regulation of the internal milieu in vascular smooth muscle and vascular tone (Reuter 1973; Hendrickx & Casteels, 1974; Blaustein, 1977; van Breemen *et al.*, 1979; Fleming, 1980; Allen & Navran, 1984). When the activity of this enzyme is inhibited, by exposure of the vessels to a K^+-free medium or preincubation with cardiac glycosides, an increase in vascular tone appears (Briggs & Shibata, 1966; Bonaccorsi *et al.*, 1977; Karaki *et al.*, 1978; Ozaki *et al.*, 1978; Ozaki & Urakawa, 1979; Mikkelsen *et al.*, 1979; Toda, 1980; Wallick *et al.*, 1982). Furthermore, the administration of K^+ to the vessels immersed in a K^+-free medium induces vasodilatation, usually due to hyperpolarization of the vascular muscle cell membrane (Webb & Bohr, 1978; 1979; Haddy, 1983; Hermsmeyer, 1983). This relaxation seems to be related to the activity of the Na^+ pump (Webb & Bohr, 1978; Haddy, 1983; Allen & Navran, 1984).

The contraction caused by the inhibition of the Na^+, K^+-ATPase may be of the myogenic and/or the neurogenic type. The predominance of one type or the other depends on the animal species and the kind of vessel (Bonaccorsi *et al.*, 1977; Toda, 1980; Hayashi & Park, 1984). In some vascular beds the myogenic component predominates; i.e., the contraction is due to the inhibition of the Na^+ pump of vascular smooth muscle cells. In others, it is essentially due to blockade of the Na^+ pump present in the adrenergic nerve

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endings. The contractions caused by ouabain in dog cerebral and peripheral arteries are of the myogenic and neurogenic type, respectively (Toda, 1980). Conversely, it has been proposed that the activation of the Na^+ pump, which hyperpolarizes vascular smooth muscle cells, is responsible for the vasodilatation caused by different drugs (Foley, 1984; Pan & Janis, 1984). In addition, disturbances in this enzyme are implicated in the pathogenesis of hypertension (Webb & Bohr, 1980; Hamlyn *et al.*, 1982).

The properties of the vascular Na^+, K^+-ATPase have been the object of numerous investigations in peripheral blood vessels, but brain vessels remain largely unexplored. Therefore, the aim of the present study was two fold: (1) to analyze in detail the mechanisms involved in the responses elicited by ouabain in cerebral arteries and, for comparison, in femoral arteries of cat, and (2) to characterize the electrogenic Na^+ pump in these vessels. For this purpose, [^{3}H]-ouabain binding to arterial membrane fractions, K^+-elicited relaxation and ouabain-sensitive $^{86}Rb^+$ uptake were measured.

Methods

Vascular reactivity studies

Cats of either sex (1.5–4 kg) were anaesthetized with sodium pentobarbitone (35 mg kg^{-1}, i.p.) and killed by bleeding. The middle cerebral and the femoral arteries were dissected into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric tension recording in an organ bath according to the method described by Nielsen & Owman (1971). The organ bath contained 6 ml of Krebs-Henseleit solution (KHS) at 37°C continuously bubbled with a 95% O_2:5% CO_2 mixture which gave a pH of 7.4. Two stainless steel pins were passed through the lumen of the arterial segment. One pin was fixed to the organ bath wall while the other one was connected to a strain gauge for isometric tension recording. The latter pin was in a parallel position with the former and was movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The isometric contraction was recorded through a force-displacement transducer (Grass FTO3C) connected to a Grass Model 7D polygraph.

A resting tension of 0.5 g and 1 g (optimal resting tone) was applied to cylindrical segments of middle cerebral and femoral arteries, respectively. This tension was readjusted every 15 min during a 90–120 min equilibration period before ionic changes in the medium or addition of drugs were made. The concentration-response curves to ouabain were determined in a cumulative manner (10^{-7} to 10^{-3} M), the glycoside being added at 5 min intervals. When phentolamine or verapamil were administered with the purpose of inhibiting the contraction caused by ouabain, they were added to the bath 10 min in advance; in other cases phentolamine was administered when the contraction caused by ouabain had reached a plateau. In order to analyze the influence of extracellular Ca^{2+} on the contractile responses elicited by ouabain, the arterial segments were exposed for 30 min to a Ca^{2+}-free KHS before its administration.

[^{3}H]-ouabain binding studies

Once isolated, the cat heart (used only for comparative purposes) and cerebral (those of the circle of Willis with their branches) and femoral arteries, were immersed in saline solution (0.9% NaCl) at 4°C. In this solution, the connective tissue and blood traces were removed and the samples were frozen at − 70°C. When about 1 g of each kind of vessel had been obtained (from several control animals used for different purposes) the binding studies were carried out according to the method of Gerthoffer & Allen (1981) with minor modifications. Briefly, the arteries were mixed and homogenized in a Sorval homogenizer in ice-cold buffer solution (1 g 20 ml^{-1}) 5 times for 10 s with 40 s rest interval. Constituents of the buffer were as follows (mM): Tris-HCl 5, sucrose 320, Na_2EDTA 0.1, pH 7.4. The homogenate was filtered through three layers of gauze and centrifuged at 800 *g* for 10 min at 4°C in a Sorval RC-5 centrifuge. The supernatant was centrifuged at 100,000 *g* for 60 min at 4°C in a Beckman L8–70 ultracentrifuge and the resultant pellet, containing the crude membranes, was resuspended in the buffer, the amount of proteins determined according to the method of Lowry *et al.* (1951) and 1 ml aliquots were placed in tubes, which were frozen at − 20°C until use.

[^{3}H]-ouabain binding was carried out in tubes containing the membranes (150 μg of protein) and 1 ml of a medium containing a buffer and a solution of adenosine triphosphate, 1.25 mM (freshly added). The composition of this buffer was (mM): Tris-HCl 50, NaCl 150, $MgCl_2$ 2.5, Na_2EDTA 1, pH 7.4. Non-specific binding was determined by addition of cold ouabain (10^{-4} M) to this medium. The tubes were then placed in a shaking water bath at 37°C and 10 min later [^{3}H]-ouabain (from 1 to 50 nM; specific activity 20.0 Ci $mmol^{-1}$) was added. The binding was stopped 60 min later by quickly cooling the tubes by immersing them in a bath at 4°C, dilution by administration of 5 ml of ice-cold buffer and rapid filtration through Whatman GF/C glass fibre filters. The filters were washed with 10 ml ice-cold buffer, dried and placed in vials containing 2 ml of Ready-Solv HP (Beckman) and radioactivity measured in a liquid scintillation counter (Beckman LS-2800). Specific binding was

calculated as the difference between the total and nonspecific binding.

$^{86}Rb^+$ uptake studies

$^{86}Rb^+$ uptake studies were done according to the method of Bukoski *et al.* (1983b), with minor modifications. Cat cerebral and femoral arteries were placed in a Petri dish containing ice-cold KHS, and once in this medium, blood traces and connective tissue were removed. The arteries were divided in segments of similar length, and then separated in groups of similar weight. Subsequently, each group of vessels was tied to a rigid nylon fibre and immersed for 30 min in a vial containing 2 ml of oxygenated (95% O_2:5% CO_2) KHS at 37°C. Afterwards, the arteries were exposed to a K^+-free medium for 15 min and then they were put in vials containing 2 ml of K^+-free, Rb^+-KHS plus $^{86}RbCl$ (10^{-6} M; specific activity: 4.68 mCi mg^{-1}) for 1, 5, 10, 15 or 30 min incubation periods. Subsequently, the tissues were washed by successive immersion in vials containing 2 ml of K^+-free, Rb^+KHS for three periods of 30 s. The arteries were then blotted, weighed, digested in 1 ml of H_2O_2 (30% w/v) by heating for 5 h at 100°C and the radioactivity measured in a similar way as previously described for binding studies. This total $^{86}Rb^+$ uptake was expressed as mmol kg^{-1} wet weight. In other experiments the ouabain-insensitive $^{86}Rb^+$ uptake was measured. The procedure was similar, except that the cardiac glycoside (2×10^{-4} M) was added to the bath 10 min before and during the incubation period with $^{86}Rb^+$. Therefore, ouabain-sensitive $^{86}Rb^+$ uptake may be estimated as the difference between the total and the ouabain-insensitive uptake.

Solutions, drugs and statistical evaluations

The composition of the KHS was (mM): NaCl 119, KCl 4.6, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1 and the disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA) 0.03. Ca^{2+}-free-KHS was made omitting $CaCl_2$ in KHS and adding 1 mM ethyleneglycol-*bis* (β-aminomethyl eter) N,N'-tetraacetic acid (EGTA). K^+-free-KHS was prepared omitting KCl and KH_2PO_4 in KHS, but replacing the latter by an equal amount of $NaH_2PO_4.H_2O$ in KHS. K^+-free, Rb^+-KHS was obtained adding 4.6 mM RbCl in K^+-free-KHS. Low-Na^+ (25 mM) solution was made replacing NaCl by isosmolar sucrose in KHS.

Ouabain was dissolved in distilled water on the day of the experiment. Stock solutions of noradrenaline, phentolamine and verapamil (10^{-2} M) were made in a saline (0.9% NaCl)-ascorbic acid (0.01%) solution, distilled water and 99.5% ethanol, respectively, and kept at − 20°C. Aliquots of these solutions were diluted just before use with KHS to obtain the desired concentrations. The ouabain and verapamil solutions were protected from the light. Reserpine was administered to the animals intraperitoneally 2 and 1 mg kg^{-1}, 48 and 24 h, respectively, before the experiments. Control and experimental responses were obtained from separate vascular preparations.

The drugs used were: ouabain octahydrate and noradrenaline bitartrate (Sigma); phentolamine methanesulphonate (Ciba-Geigy); verapamil hydrochloride (Knoll); reserpine (NBC); [^{3}H]-ouabain and ^{86}rubidium chloride (New England Nuclear). Results were expressed as means ± s.e.means. Deviations from the mean were statistically analyzed using Student's *t* test; a probability value of less than 5% was considered significant.

Results

Vascular reactivity

Ouabain induced transient contractile responses in cat middle cerebral artery, that declined to the resting level within 15 min (Figure 1). The cumulative administration of ouabain at 5 min intervals between each concentration, induced concentration-dependent contractions up to 3×10^{-6} M; higher concentrations caused relaxation. However, ouabain induced concentration-dependent contractile responses in cat femoral arteries (Figure 1). Contractions evoked by ouabain in cerebral vessels were unaffected by phentolamine (3×10^{-6} M) or reserpine pretreatment, whereas they were abolished by verapamil (3×10^{-6} M) or Ca^{2+}-omission (Figure 2). Nevertheless, the vasoconstriction induced by this drug in the femoral artery was diminished by phentolamine (3×10^{-6} M), pretreatment with reserpine or incubation in a Ca^{2+}-free medium, but not by verapamil (3×10^{-6} M) (Figure 2). In addition, the administration of phentolamine (10^{-6} and 3×10^{-6} M) at the peak of the contraction produced by ouabain (10^{-4} M) induced concentration-dependent vasodilatation (Figure 1).

On the other hand, the omission of K^+ from the medium, in order to inhibit Na^+, K^+-ATPase, induced a small and transient contraction in both kinds of arteries. The subsequent administration of K^+ (7.5 mM) to these vessels previously contracted by noradrenaline (10^{-5} M) induced vasodilatation (Figure 3). This response in cerebral and femoral arteries was 236 ± 10 and 486 ± 30 mg, respectively. The presence of ouabain (10^{-4} M) abolished this relaxation, and there was then a small contraction (155 ± 13 and 307 ± 29 mg, respectively).

The exposure of the arteries to a medium containing Na^+ (25 mM) produced a large but transient increase in tension. The maximal response reached in cerebral

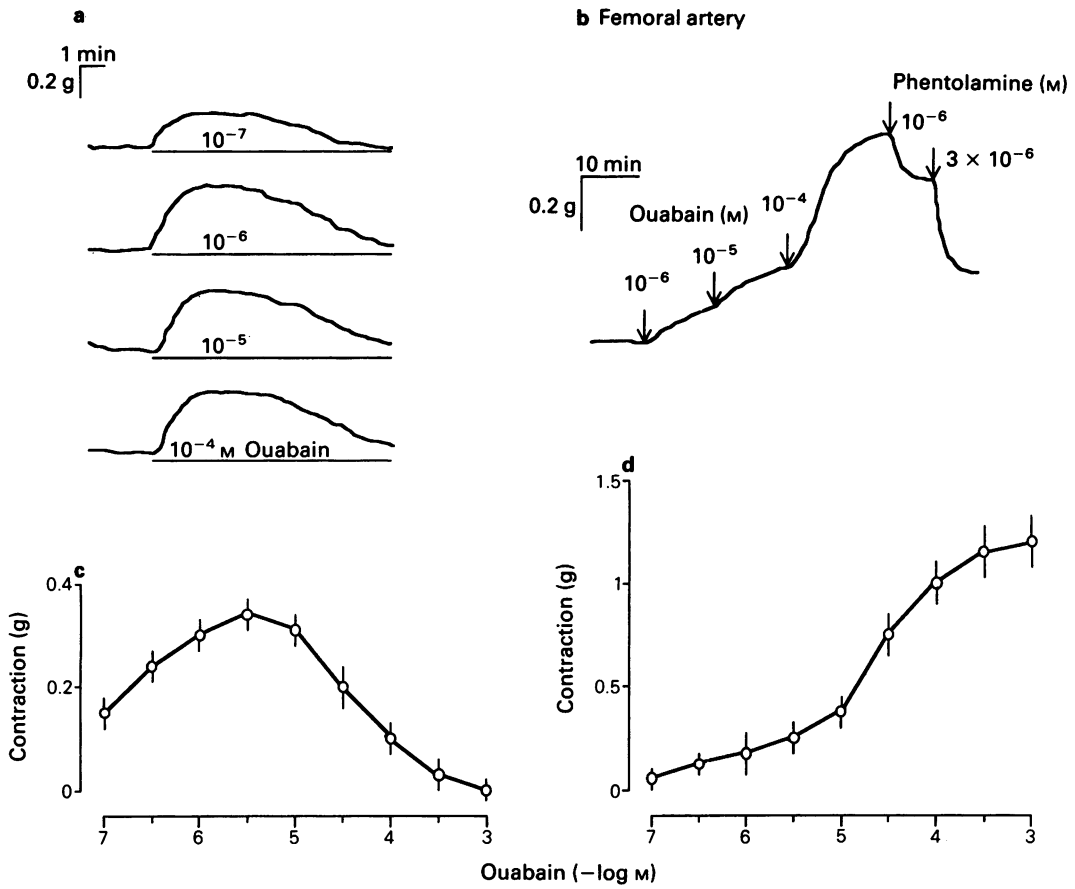

Figure 1 Recordings showing the isometric tension responses induced by different concentrations of ouabain in segments of cat middle cerebral arteries (a) and by cumulative administration in femoral arteries (b). In the latter the effect of phentolamine added at the peak of the contraction is also shown. Below: concentration-response curves for ouabain in cerebral (c) and femoral arteries (d). The application of ouabain was made at 5 min intervals. Values are means with s.e.means shown by vertical lines ($n = 13$).

($n = 5$) and femoral ($n = 4$) arteries was 1,310 ± 230 and 2,130 ± 360 mg, respectively. The contraction in brain vessels returned to the resting level within 15 min, but it persisted at 1,210 ± 160 mg in femoral arteries. In this medium, ouabain did not produce any further contraction in either kind of artery.

[³H]-ouabain binding

Since it was difficult to obtain a sufficient amount of cerebral and femoral arteries to make a complete study of [³H]-ouabain binding, an incubation equilibrium time of 60 min and 150 μg of protein in the tubes were chosen, in accordance with previous studies on different smooth muscles (Gerthoffer & Allen, 1981; Bukoski *et al.*, 1983b; Adams *et al.*, 1983).

The specific [³H]-ouabain bound to membranes of cerebral and femoral arteries as a function of [³H]-ouabain concentration, as well as the Scatchard analysis of the data are shown in Figure 4. Maximum number of binding sites (B_{max}) and the dissociation constant (K_D) were: 303.5 fmol mg^{-1} of protein and 5 nM in the case of cerebral arteries and 74 fmol mg^{-1} protein and 8.4 nM in the case of femoral ones. In cat heart membranes the K_D and B_{max} values, also determined, by Scatchard analysis, were 14.2 nM and 6,560 fmol mg^{-1} protein, respectively.

$^{86}Rb^+$ uptake

Cat cerebral and femoral arteries showed a time-dependent $^{86}Rb^+$ uptake, the equilibrium being rea-

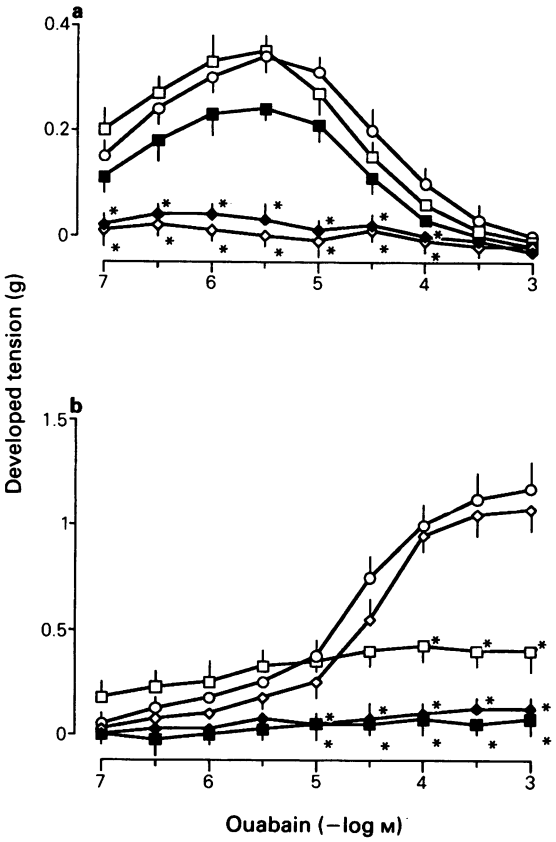

Figure 2 Effect of 10 min preincubations with phentolamine 3×10^{-6} M, (□), verapamil 3×10^{-6} M, (◇); exposure for 30 min to a Ca^{2+}-free solution (◆), or reserpine pretreatment (■) on the control contraction-response curve for ouabain (○) of cat middle cerebral (a) and femoral arteries (b). The administration of ouabain and symbols as in Figure 1. The number of arterial segments used for each curve ranges from 4 to 19 ($^*P < 0.05$).

ched at 15 min. The administration of ouabain (2×10^{-4} M) 10 min before and during the incubation period significantly reduced the $^{86}Rb^+$ uptake at 10 and 15 min (Figure 5).

Discussion

The present experiments show that ouabain induced contraction of cerebral and femoral arteries of cat, although the increase in tension was not sustained in the former. These results indicate that the properties of the electrogenic Na^+ pump in each type of artery may be different. In other vascular preparations, ouabain also induced increases in tension (van Breemen *et al.*, 1979) and in some of them the response was not sustained (Palatý, 1980; Adams *et al.*, 1983).

Phentolamine and reserpine pretreatment reduced the contraction caused by ouabain in femoral arteries; these drugs produce a blockade of α-adrenoceptors and a depletion of vascular catecholamines in these vessels, respectively (Marín *et al.*, 1982). These findings suggest that the response elicited by ouabain in this preparation is, in great part, mediated by noradrenaline release from perivascular adrenergic nerve endings (neurogenic effect), probably as a result of Na^+ pump inhibition of the sympathetic terminals (Wallick *et al.*, 1982). However, in brain vessels, the same response seems to be due to a direct action on vascular smooth muscle cells (myogenic effect). Similar results have been found in dog cerebral and peripheral arteries (Toda, 1976). Other authors have also observed that ouabain-induced contraction in different peripheral arteries is of the neurogenic type (Broekaert & Godfraind, 1973; Palatý, 1980; Adams *et al.*, 1983; Aarhus *et al.*, 1983). It is interesting to note that the exposure of the vessels preincubated with [^{3}H]-noradrenaline to ouabain (10^{-4} M) for 30 min produced noradrenaline secretion from cat cerebral, but not femoral, arteries (Marin *et al.*, 1986). However, on increasing the time of exposure to ouabain from 30 to 60 min, a small, but significant, increase in [^{3}H]-noradrenaline secretion in the latter vessels is produced after 30 min (results not shown). The greater noradrenaline release induced by ouabain in cerebral vs. femoral arteries is consistent with the fact that the noradrenaline content of the former is 8 times greater than that of the latter vessels (Marín *et al.*, 1982). However, since the opposite occurs with the response of these arteries to noradrenaline (Marín *et al.*, 1982), it may be inferred that the contribution of the released noradrenaline to the total contraction caused by ouabain in cerebral arteries is small in comparison with the myogenic component.

The exposure of both kinds of arteries to a Ca^{2+}-free medium decreased the contraction caused by ouabain. These results point to the dependence of the response caused by the cardiac glycoside on the extracellular Ca^{2+}, which agrees with the findings obtained in different vascular beds (Briggs & Shibata, 1966; van Breemen *et al.*, 1979; Toda, 1980). In addition, verapamil diminished the increase in tension elicited by ouabain only in cerebral arteries, which suggests that Ca^{2+} enters into the vascular smooth muscle cells via Ca^{2+} antagonist-sensitive channels, as it occurs in dog cerebral arteries (Toda, 1980). It has been reported that the mechanism by which Ca^{2+} enters the vascular smooth muscle cells, following Na^+, K^+-ATPase inhibition, is by a Na^+-Ca^{2+} exchange system (van Breemen *et al.*, 1979; Hermsmeyer, 1983; Allen & Navran, 1984), which appears to be insensitive to Ca^{2+}

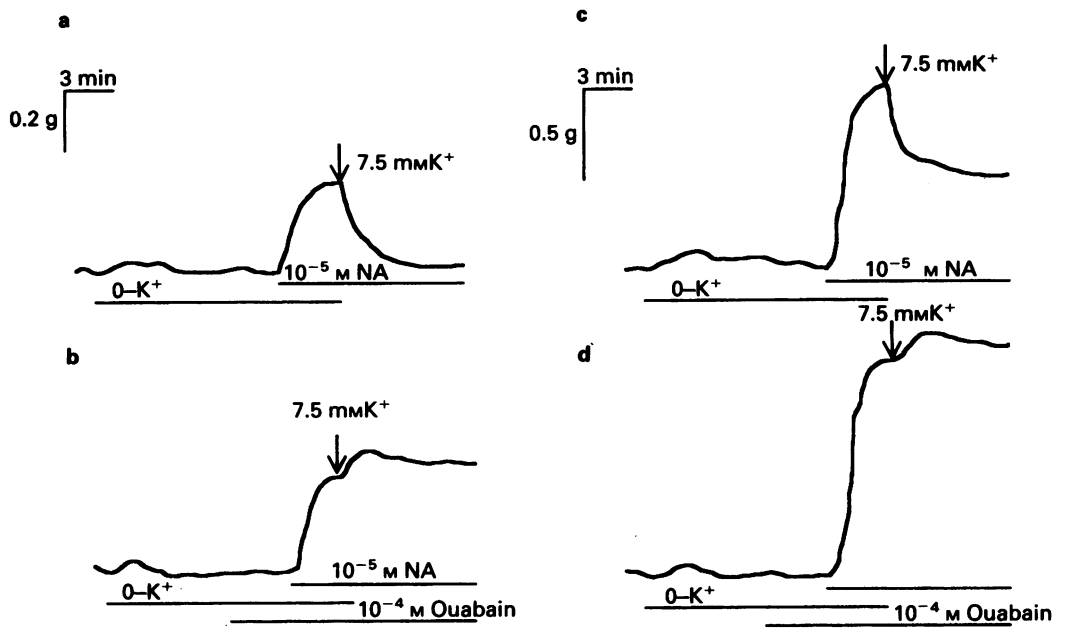

Figure 3 Typical records showing the effect of ouabain on K^+-induced relaxation in cat middle cerebral (a,b) and femoral (c,d) arteries. Following a period of exposure to a K^+-free solution, noradrenaline (NA) was added to the bath and subsequently 7.5 mM K^+ was administered. The time of exposure to different drugs and zero K^+ is indicated by horizontal bars. The experiment lasted 25 min. At least 4 experiments of each type were carried out.

antagonists (Ozaki & Urakawa, 1979; van Breemen *et al.*, 1979). Therefore, the Ca^{2+} influx into smooth muscle cells of cerebral arteries caused by ouabain could be different from Na^+-Ca^{2+} exchange. The Na^+ pump inhibition seems to produce a small depolarization of the smooth muscle fibres (Reuter, 1973; Hendrickx & Casteels, 1974; Fleming, 1980). This could facilitate, in these vessels, the Ca^{2+} entry into the cells through voltage-sensitive Ca^{2+} channels, which are very sensitive to Ca^{2+} antagonists (Bolton, 1979). It is interesting to note that these channels seem to be preactivated in cat cerebral arteries, since the administration of Bay-K8644, a Ca^{2+} agonist, produced a concentration-dependent contraction by specific facilitation of Ca^{2+} entry through these channels. However, this agonist caused a response in femoral arteries only when a partial depolarization was produced with 15 mM K^+ (Salaices *et al.*, 1985). Furthermore, Harder (1980) observed that: (1) cat cerebral arteries have a greater resting membrane potential than peripheral arteries, and (2) ouabain and K^+ induce a major depolarization in the former vessels. All these data also support the hypothesis that ouabain might produce Ca^{2+} influx through the above mentioned channels.

The release of catecholamines induced by cardiac glycosides in different tissues, including these arteries (García & Kirpekar, 1973; Esquerro *et al.*, 1980; Wakade, 1981; Marín *et al.*, 1986), seems to be dependent on extracellular Ca^{2+}. Therefore, the suppression of the response evoked by ouabain in femoral arteries by Ca^{2+} omission is probably due to the reduction of both the noradrenaline release and the contraction caused by that secreted. In addition, it has been reported that Ca^{2+} channels present in the sympathetic nerves of these vessels (Marín *et al.*, 1985) and other tissues (Haeusler, 1972; Göthert *et al.*, 1979) possess a low sensitivity to Ca^{2+} antagonists. The same appears to occur with receptor-operated Ca^{2+} channels involved in the contraction induced by the noradrenaline released (Bolton, 1979). These facts could explain the lack of effect of verapamil on the responses elicited by ouabain in femoral arteries.

When the arteries were immersed in a K^+-free medium a small and transient contraction appeared. In other vessels, it has been reported that this response ranges from no increase in tension to a marked contraction (Bonaccorsi *et al.*, 1977; Karaki *et al.*, 1978; Lang & Blaustein, 1980; Hayashi & Park, 1984), suggesting different Na^+ pump activities in the vascular beds. These contractions are due to Na^+ pump inhibition produced by K^+-omission (van Breemen *et*

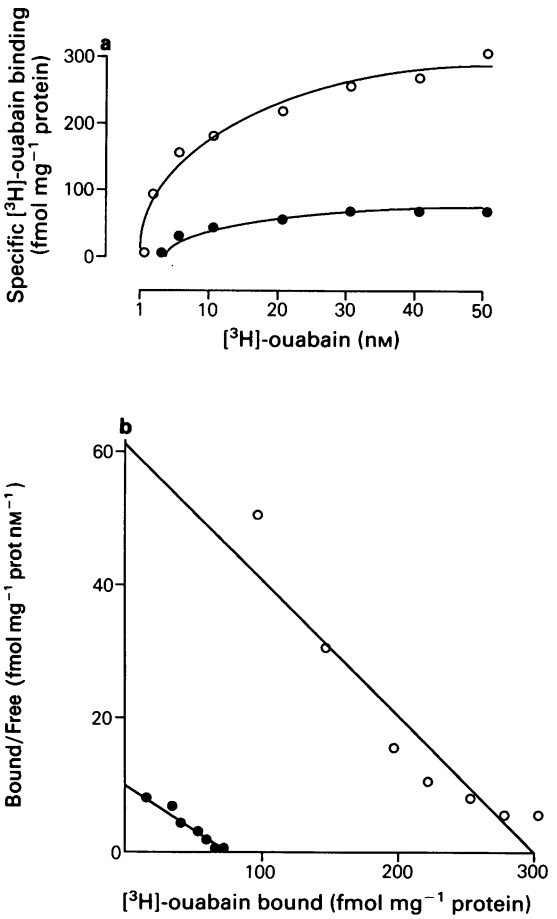

Figure 4 (a) Representative concentration-dependent [^{3}H]-ouabain binding to crude membranes from cat cerebral (○) and femoral (●) arteries. Specific [^{3}H]-ouabain binding is plotted vs. increasing concentrations of administered [^{3}H]-ouabain. (b) Scatchard analysis derived from specific binding data of the results showed in the top figures. The slope of the plot was determined by linear regression analysis. Cerebral arteries: $K_D = 5$ nM, $B_{max} = 303.5$ fmol mg^{-1} protein, $r = 0.92$. Femoral arteries = $K_D = 8.4$ nM, $B_{max} = 74$ fmol mg^{-1} protein, $r = 0.97$.

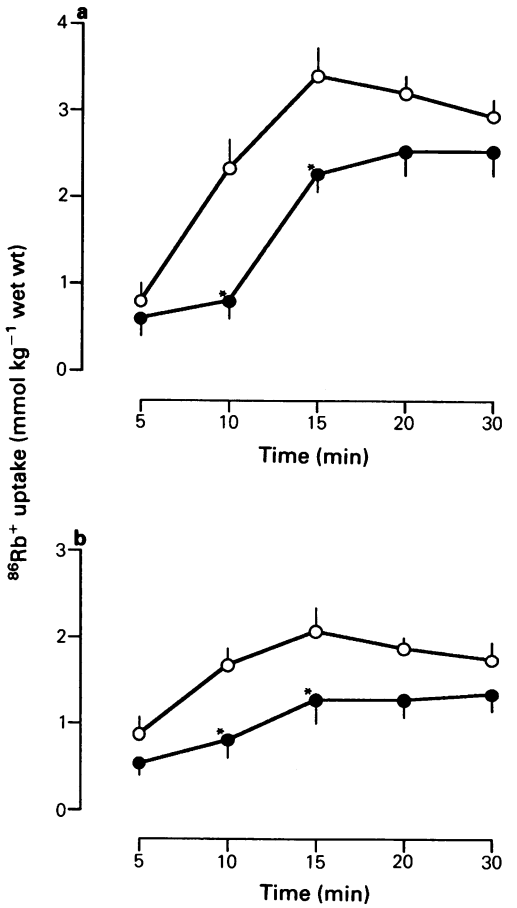

Figure 5 Total $^{86}Rb^+$ uptake (○) and ouabain-insensitive $^{86}Rb^+$ uptake (●) as a function of incubation time in cat cerebral (a) and femoral (b) arteries exposed to a medium without or with ouabain (2×10^{-4} M) respectively. Each point represents mean of 3–10 paired experiments with s.e.mean shown by vertical lines ($^*P < 0.05$).

al., 1979; Haddy, 1983; Hermsmeyer, 1983). The subsequent addition of K^+ elicited vasodilatation which was blocked by ouabain. This finding is similar to results obtained with other vascular preparations, and it is produced by Na^+ pump activation (Bonaccorsi *et al.*, 1977; Webb & Bohr, 1978; Murray & Sparks, 1978). In addition, Webb & Bohr (1978) suggested that this relaxation may be an indicator of Na^+, K^+-ATPase activity. The vasodilatation obtained in cerebral arteries was greater than that found in femoral arteries, since the vascular tone caused by noradrenaline was abolished by K^+ addition in brain vessels while it was only reduced to approximately 50% in femoral vessels. Similar findings were obtained in dog cerebral and peripheral arteries (Toda, 1976).

In order to decrease the activity of the Na^+, K^+-ATPase, the concentration of Na^+ in the medium was reduced. This caused a transient increase of vascular tone and abolished the contraction induced by ouabain in both kinds of arteries. Other authors have reported that exposure of tissues to a Na^+-free solu-

tion suppresses the response evoked by ouabain and K^+ reduction, as well as the vasodilatation elicited by subsequent K^+ addition to the medium (Toda, 1974; 1978; 1980; Linden & Brooker, 1980), procedures which are known to modify the activity of Na^+, K^+-ATPase.

In intact vascular smooth muscle, the Na^+ pump functioning can also be tested by a direct study of the pump turnover by means of ouabain-sensitive $^{86}Rb^+$ (a K^+ congener) uptake (Bukoski *et al.*, 1983a,b). Our results show that the total and the ouabain-sensitive $^{86}Rb^+$ uptake was higher in cerebral than in femoral arteries. These data suggest a greater Na^+ pump activity in cerebral than in femoral arteries, which is in agreement with the results obtained with K^+-evoked relaxation.

It has been reported that [^{3}H]-ouabain binding can be used to characterize the Na^+ pump in smooth muscle preparations (Deth & Lynch, 1980; Gerthoffer & Allen, 1981). The results obtained in the binding experiments showed that specific [^{3}H]-ouabain binding was saturable in both kinds of arteries, and pointed to the existence of a homogeneous population of binding sites determined by the Scatchard analysis. Similar results have been obtained in membranes of other vessels (Wallick *et al.*, 1982; Fox *et al.*, 1983). The K_D values (Figure 4), a measure of the affinity of Na^+, K^+-ATPase for ouabain, indicate that the enzyme present in both kinds of arteries has a relative and similar high affinity for the glycoside. The K_D values obtained in dog mesenteric artery microsomes range from 2 to 9 mM (Wallick *et al.*, 1982; Adams *et al.*, 1983) and in those of bovine aorta were around 4 nM (Fox *et al.*, 1983), i.e., similar to those found in the present study. In addition, the B_{max} value in membranes of cerebral arteries was greater than that obtained in the same fractions of femoral arteries; this suggests that cerebral arteries possess a higher density of ouabain binding sites. The B_{max} values in dog mesenteric artery (Wallick *et al.*, 1982; Adams *et al.*, 1983) and bovine aorta microsomes (Fox *et al.*, 1983) range from 2 to 6.24 pmol mg^{-1} protein, i.e. greater than those found in microsomes of cerebral and femoral arteries. These differences between our results and those found in dog mesenteric artery and bovine aorta could be due either to a higher density of Na^+ pump sites in the latter vessels or simply to methodological differences. It is necessary to note that the vessels that we used are very small, particularly the cerebral arteries, and that the mixture of membranes obtained (from smooth muscle, adventitia and endothelium) is probably different from that obtained from dog mesenteric artery and bovine aorta. In addition, the K_D value obtained in cat heart membranes was of a similar order to those found in cerebral and femoral arteries, while the B_{max} was greater. These results suggest that the affinity of the enzyme for ouabain is similar in these kinds of tissues, but the number of Na^+ pump sites in vascular smooth muscle is fewer than in cardiac muscle. Other authors have also found similar differences between the heart muscle and smooth muscle (Gerthoffer & Allen, 1981; Wallick *et al.*, 1982; Adams *et al.*, 1983).

In conclusion, our results indicate that cerebral arteries were more sensitive to ouabain than femoral arteries. The contraction elicited by the cardiac glycoside in cerebral arteries seems to be caused by Na^+, K^+-ATPase inhibition of vascular smooth muscle cells (myogenic effect), while in femoral arteries it was due to noradrenaline release from adrenergic nerve endings (neurogenic effect). Both the activity of Na^+, K^+-ATPase and the density of Na^+ pump sites were greater in cerebral than in femoral arteries, while the affinity of the enzyme for ouabain was similar in both cases.

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