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Ouabain-insensitive, halide-sensitive Tl^+ uptake by canine iliac arteries

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Ouabain-insensitive Tl^+ uptake by canine iliac arteries was studied. A significant fraction was halide-sensitive, with Br^- substituting well for Cl^-. The halide-sensitive component was inhibited by diuretics (MK196, bumetanide), PCMBS, low temperatures and external cations K^+, Rb^+. External but not internal Na^+ was necessary for the uptake process. The process was not sensitive to disulphonic stilbenes. The halide-sensitive uptake appears to represent the operation of a ($Na^+/K^+/Cl^-$)-cotransport process in arteries.

Introduction

In canine iliac arteries, total Tl^+ uptake can be partitioned into an ouabain-sensitive and an ouabain-insensitive component. The former component which accounts for 70% of total uptake by Na^+-loaded segments was modulated in a predictable manner by manoeuvers known to alter the activity of the Na^+ pump and thus represented inward Tl^+ movement on the K^+ limb of the classical (Na^+-K^+) exchange system (the Na^+ pump). We sought to define further the characteristics of the ouabain-insensitive component and considered the possibility that this could represent part of a (K^+/Cl^-)- or ($Na^+/K^+/Cl^-$)-cotransport system. We found that replacement of chloride in the bathing solutions by nitrate significantly reduced the ouabain-insensitive component and have thus adopted an operational definition of a halide-sensitive component as being the difference in Tl^+ uptake in halide and nitrate solutions, ouabain being present in both. In this study, we report some of the characteristics of ouabain-insensitive, halide-sensitive Tl^+ uptake by canine iliac arteries.

Abbreviations: DIDS, diisothiocyanostilbene-2,2′-disulphonic acid; PCMBS, *p*-chloromercuribenzenesulphonic acid.

Materials and Methods

All studies were carried out using segments of canine iliac arteries obtained from adult mongrel dogs that had been euthanised with sodium pentabarbitone (100 mg/kg) (see Ref. 1). After removal, the arteries were dissected in normal K^+-containing solutions that were continuously bubbled with 95% O_2/5% CO_2 and incubated subsequently in the same solutions for 60 min at 37°C. Following this recovery period, the tissues were transferred to K^+-free nitrate solutions for a further incubation period of 90 min. Following this preincubation period, the tissues were transferred to incubating solutions containing $^{204}Tl_2SO_4$ with $TlNO_3$ in varying amounts (0.125–5 mM). After a 20 min incubation period with isotopes, the tissues were removed, quickly blotted and weighed into vials. They were then either dried in an air-oven to constant weight (in experiments where dry weights were measured) or were digested with 1 ml Protosol and 100 μl H_2O_2 in an air-oven at 70°C. The digested tissues were processed for counting in a Beckman liquid scintillation counter [1]. Aliquots

of the incubation media were taken and processed to estimate total added counts. The uptake of Tl^+ was expressed as μmol/g wet or dry weight.

The above protocol was a modification of the one used in the earlier study [1]. In K^+-free solutions, the Na^+ pump is inhibited and readmission of K^+ or Tl^+ sets the pump functioning at a maximal rate, yielding a better signal. In the current study, we followed the procedure of preincubating in K^+-free solution since we noted in preliminary experiments that the ouabain-insensitive Tl^+ uptake was higher when tissues were preincubated in K^+-free solution than in tissues that had been preincubated in normal K^+-containing solutions (0.147 compared to 0.092 μmol/g per min). As pointed out (in introduction), we operationally defined halide-sensitive uptake by comparing uptakes in halide and nitrate solutions and, therefore, we even preincubated the tissues in nitrate solutions.

To summarise, the experimental design was as follows: Arteries were preincubated in K^+-free NO_3 solutions for 90 min, following which they were transferred to incubation vials containing either K^+-free NO_3 or K^+-free halide solutions with $TlNO_3$, $^{204}Tl_2SO_4$ and ouabain at 10^{-5} M being present in all vials. The uptake in the nitrate solution was taken as the residual so that ouabain-insensitive halide-sensitive Tl^+ uptake was by definition: Tl^+ uptake in halide solutions minus Tl^+ uptake in nitrate solutions. Hereafter, we will refer to this component as the halide-sensitive component (omitting repetition of the qualifying expression ouabain-insensitive).

In most cases, we pooled data from experiments done on different days and have chosen to 'normalise' our data with respect to appropriate control values. In each instance, the choice of the control value is stated and this method of expressing our results helps emphasise the points we wish to make.

Results

Linearity of uptake. The halide-sensitive uptake was linear for at least 30 min as shown in Fig. 1. The results shown are those of a single experiment. From four such experiments, we estimated that the rate of uptake was 0.042 ± 0.009 μmol/g per min.

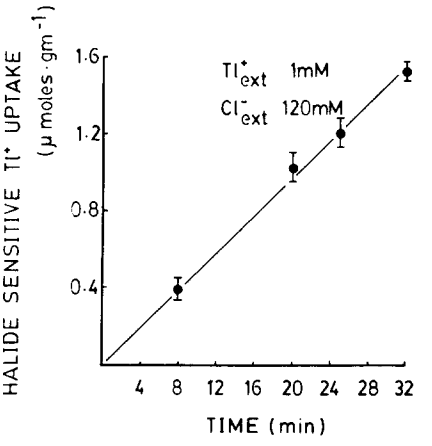

Fig. 1. The ouabain-insensitive, halide-sensitive uptake of Tl^+ was linear for at least 20 min. (Data are taken from a single experiment).

In all subsequent experiments, incubations were carried out for 20 min with the isotope.

Dependence on external chloride. The halide-sensitive uptake of Tl^+ showed a significant dependence on external chloride, being saturated at concentrations close to the concentrations normally present in physiological salt solutions (120 mM). The apparent K_m for this process would be in the order of 45 mM external chloride (see Fig. 2). As noted, in the above experiments, the concentration of external Tl^+ was kept constant at 1 mM.

Dependence on external Tl^+. To determine whether the concentration of Tl^+ used in the above experiment was optimal, we studied the halide-sensitive uptake of Tl^+ as a function of external Tl^+ concentration. The results are shown in Fig. 3, where the uptakes are normalised to the uptake of Tl^+ at an external Tl^+ concentration of 1 mM. The curve shows a peak at 2 mM external Tl^+ concentration and tends to decline at higher concentrations. In all subsequent experiments (except where stated), the concentration of Tl^+ in the external solution was kept at 1 mM.

Effect of other halides. In the above experiments, we had studied the uptake of Tl^+ in solutions containing Cl^- as the predominant halide. To determine whether other halides could substitute for chloride, we attempted to measure the uptake of Tl^+ in the presence of Br^- and I^-. Unfortunately, thallous iodide precipitated out of

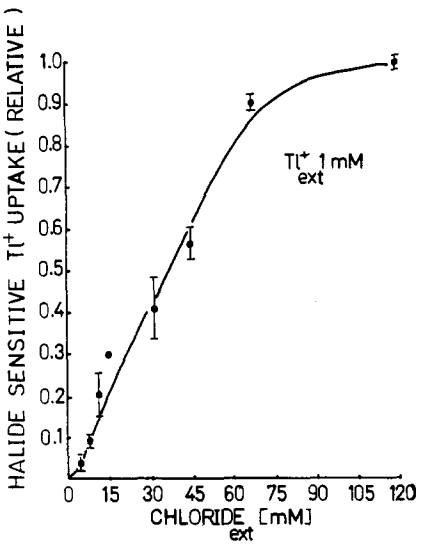

Fig. 2. Halide-sensitive Tl^+ uptake was a saturable function of external Cl. Uptakes were measured for 20 min and all values are expressed relative to the uptake observed with 120 mM external Cl. (Composite curve from seven experiments.)

the solution and so uptakes could not be measured. With bromide replacing chloride, a precipitate was also noted if the thallium concentrations were higher than 0.5 mM, so uptakes were measured with Tl^+ concentrations of 0.125 mM. This was clearly suboptimal as judged from the earlier

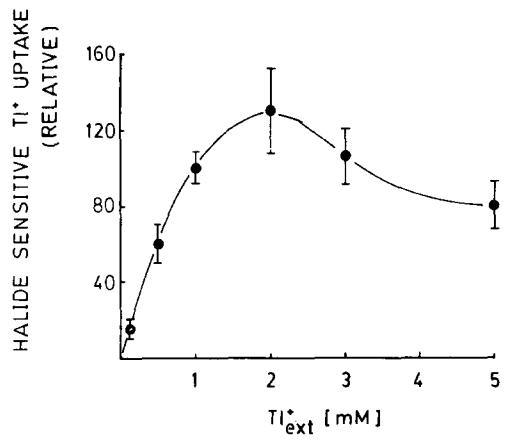

Fig. 3. The halide-sensitive uptake of Tl^+ as a function of external Tl^+. Uptakes were measured for 20 min and all values are expressed relative to the uptake observed with 1 mM external Tl^+. (Composite curve from five experiments.)

experiments. Nevertheless, we found that the uptake was significantly higher in bromide- than in chloride-containing solutions (Fig. 4) at every concentration of the halide tried. In the figure, all values have been normalised to the Tl^+ uptake observed in 120 mM Cl^-. We attempted to determine the effects of other anions on Tl^+ uptake. Unfortunately, the thallous salts of a number of anions are insoluble and thus, precipitates formed in solutions. Acetate, as an anion substitute, failed to stimulate Tl^+ uptake. In the presence of acetate, ouabain-insensitive, Tl^+ uptake was $94.0 \pm 5\%$ of the uptake in the presence of nitrate.

Effects of the diuretic MK196. A number of diuretics, e.g. furosemide, have been shown to inhibit $(Na^+/K^+/Cl^-)$-cotransport in a variety of cells and tissues. We tested the effects of the diuretic MK196 on halide-sensitive Tl^+ uptake in the presence of nitrate and Cl^- and subtracted the appropriate residual uptake. In Fig. 5, all values are related to halide-sensitive uptake in the absence of the drug. Clearly, the diuretic markedly reduces the uptake of Tl^+, though even with concentrations as high as 1.0 mM, 10% of the uptake temained insensitive to the diuretic. Br^--sensitive

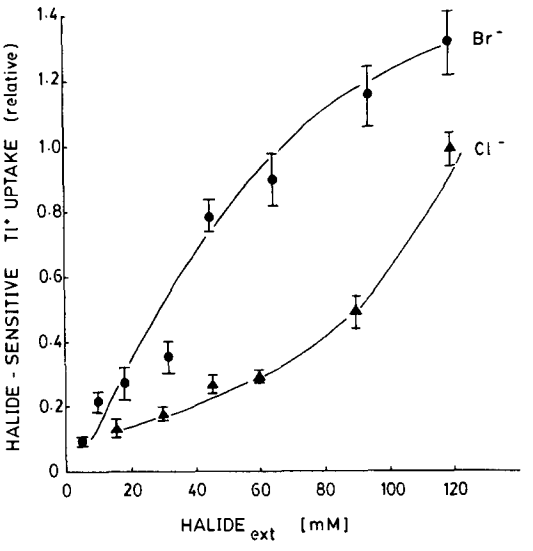

Fig. 4. Comparison of the uptake of Tl^+ in the presence of external Cl and bromide. The Tl^+ concentration was reduced to 0.125 mM to prevent precipitation of Tl-Br. Here again uptakes were measured for 20 min and all values expressed relative to the uptakes measured with external Cl of 120 mM. (Composite curve from five experiments.)

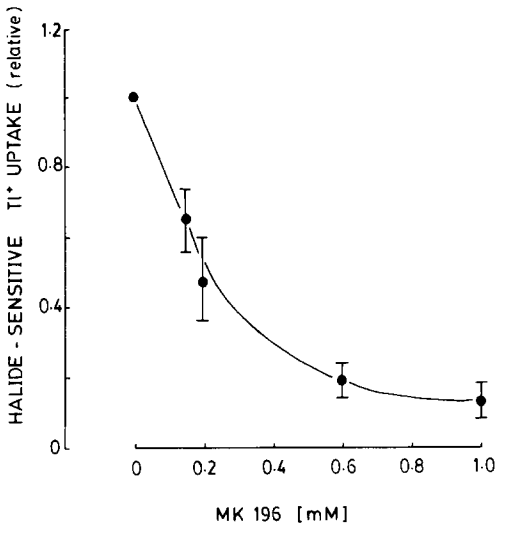

Fig. 5. The effect of the diuretic MK196 on halide-sensitive Tl^+ uptake. The values are expressed relative to the uptakes observed in the absence of the drug. (Data are taken from three experiments.)

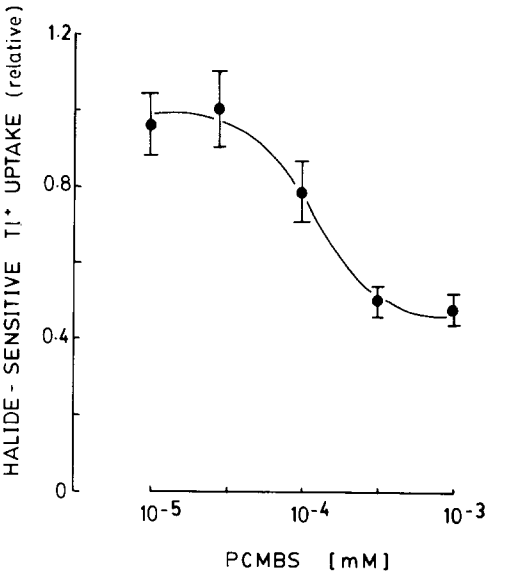

Fig. 6. The effect of the -SH reagent PCMBS. Here again the values are expressed relative to the uptakes observed in the absence of the -SH reagent. (Data are taken from three experiments.)

Tl^+ uptake was similarly affected. The diuretic, bumetanide, also inhibited Cl^--sensitive Tl^+ uptake.

Effects of the -SH reagent, PCMBS. The -SH reagent, PCMBS present in the incubation media, reduced halide-sensitive uptake of Tl^+. Here again, the effects of the drug were compared in NO_3^- and Cl^- solutions (Fig. 6).

Effects of other cations. The effects of external Rb^+, K^+ and Li^+ on the uptake of Tl^+ were studied. We tested the effects of 30 mM Rb^+, K^+ or Li^+ used as the nitrate salts and, Cl^- concentrations being reduced to 90 mM. This would not have seriously affected Tl^+ uptake. At any rate, the values are normalised to control uptakes in the presence of 90 mM Cl^-. In three separate experiments, we found that with an external Tl^+ concentration of 1 mM, Rb^+ and K^+ inhibited uptakes by $82.9 \pm 3.3\%$ and $86.7 \pm 1.7\%$, respectively. Li^+, on the other hand, appeared to stimulate uptake but the effects were not statistically significant (by $18 \pm 22\%$ in three experiments). We repeated the above experiments using Br^- as the anion and reducing the concentration of Tl^+ to 0.125 mM. Once again, Rb^+ and K^+ inhibited Br^--sensitive Tl^+ uptake by $86.0 \pm 3.0\%$ and $87.0 \pm 1.7\%$, respectively in three experiments, whereas Li^+ appeared to produce a slight stimulation (6%). Thus, both Rb^+ and K^+ inhibited halide-sensitive Tl^+ uptake. External Li^+ at least used in a concentration of 30 mM did not appear to inhibit the process.

Effects of Na^+ removal on halide-sensitive Tl^+ uptake. To determine whether total replacement of external Na^+ by lithium would alter halide sensitive Tl^+ uptake, we did the following experiment. Na^+-loaded tissues were incubated for 6 min in one of the following solutions: NaCl-Krebs, $NaNO_3$-Krebs, LiCl-Krebs, $LiNO_3$-Krebs, and the ouabain-insensitive uptakes of Tl^+ were monitored. The shorter incubation period was chosen, to exclude the possibility that sufficient Na^+ would have leaked out into the lithium solution. In two separate experiments, incubation in lithium solutions reduced halide-sensitive Tl^+ uptake by 48% and 63%, respectively. Clearly then, total replacement of external Na^+ by lithium does reduce uptake of Tl^+. To determine whether internal Na^+ was necessary, we repeated the above experiments using lithium-loaded tissues. When lithium-loaded tissues were incubated in Na^+-containing solutions, the halide-sensitive uptake was comparable

to those observed with Na^+-loaded segments (in fact, a slight stimulation being noted). As with the Na^+-loaded segments, removal of external Na^+ significantly reduced halide-sensitive uptake. Clearly then, internal Na^+ did not seem critical for the operation of the uptake process.

Effect of disulphonic stilbenes (DIDS): DIDS used in fairly high concentrations (0.2 mM) failed to produce any effect on halide-sensitive (Tl^+ uptake. This could imply the lack of involvement of a $Cl^-HCO_3^-$ exchange process in the uptake of Tl^+ or the lack of suitable binding sites for DIDS. Total replacement of HCO_3^- in the external medium did not alter uptake either.

Effect of temperature. Lowering the circulation temperature significantly reduced halide-sensitive Tl^+ uptake, in some experiments to negligible levels. In one representative experiment, there was no measurable halide-sensitive uptake at 4°C but at 37°C, the uptake measured was 0.104 μmol/g per min. We have not, however, attempted to characterise in detail, the temperature dependence of the process and estimate activation energies.

Effect of chloride removal on tissue water contents. In other tissues, the ($Na^+/K^+/Cl^-$)-cotransport system has been suggested to control cell volume [2]. The regulation of cell volume in smooth muscle is a difficult problem [3,4] and there appears to be both species and tissue variations. In canine iliac arteries, inhibition of the Na^+ pump does not lead to tissue swelling [3] and it is thus possible that an alternative mechanism could regulate cell volume. We carefully estimated water contents of tissues that had been incubated in solutions of differing ionic compositions and noted as shown in Table I that neither the presence of ouabain nor the removal of Cl^- had any significant effects on water contents estimated from wet weight/dry weight ratios.

TABLE I

TISSUE WATER CONTENTS OF INCUBATED ARTERIES

Values are expressed as H_2O g/kg wet wt. and refer to means ± S.D. of five replicates in each group. None of the values was statistically significantly different from the others. Thus, neither the presence of ouabain nor removal of Cl^- alters significantly the water contents of incubated arteries.

$[Cl_{ext}]$ (mM)	− Ouabain	+ Ouabain
130	708.4 ± 6.84	714.0 ± 8.4
43.3	704.4 ± 8.2	707.0 ± 7.03
11.8	704.4 ± 7.02	705.8 ± 8.7
0	710.8 ± 6.6	705.5 ± 6.13

Discussion

The purpose of the present study was to characterise ouabain-insensitive Tl^+ uptake by canine iliac arteries and test the hypothesis that this uptake represented the operation of a ($Na^+/K^+/Cl^-$)-cotransport process, such systems having been described in a variety of cells and tissues [2,5–11].

The positive findings of this study were as follows: (1) A significant portion of ouabain-insensitive Tl^+ uptake was reduced by replacing Cl^- with NO_3^-. (2) Br^- substituted well for Cl^- in enhancing ouabain-insensitive Tl^+ uptake but other anions such as acetate were ineffective. (3) The uptake was significantly reduced by the diuretic MK196 and bumetanide as well as the -SH reagent, PCMBS. (4) External cations particularly Rb^+ and K^+ in concentrations of 30 mM reduced markedly the uptake of Tl^+. (5) Total replacement of external Na^+ by Li^+ significantly reduced halide-sensitive Tl^+ uptake but internal Na^+ did not appear to be critical with Li^+ being able to substitute effectively. (6) The uptake process was reduced by low temperatures. (7) The uptake was unaffected by disulphonic stilbene derivatives.

The simplest explanation for the above observations is that part of the ouabain-insensitive Tl^+ uptake represents the operation of a ($Na^+/K^+/Cl^-$)-cotransport system. Internal Na^+ appears to be less critical since the operation of a halide-sensitive Tl^+ uptake could be demonstrated even in lithium-loaded tissues, provided external Na^+ was present. Detailed analyses on the relative dependence on internal and external Na^+ have not as yet been done.

Other possibilities remain to be tested. We have argued following earlier reports [11] that the decline in Tl^+ uptake at high concentrations arises from the lower solubility of Tl^+ salts, but it is possible that at higher concentrtions, Tl^+ could bind to the Na^+ site forming an ineffective complex. Again, the differences in uptakes observed

with Cl^- and Br^- may not reflect merely differences in affinity but may be related to other factors such as increased permeability of a Tl-Br complex in comparison with a Tl-Cl one. These neutral complexes may dissociate in the cell and the resultant Tl^+ being less permeable would tend to accumulate [12]. This could be one explanation for the observed uptake against a concentration gradient. Alternatively, Tl^+ could be sequestered and bound to negatively charged intracellular sites.

$(Na^+/K^+/Cl^-)$-cotransport systems have now been demonstrated in a variety of cells and tissues [5–11]. In early studies, human red cells were found to exhibit ouabain-resistant influxes of Na^+ and K^+ that appeared to be interdependent, and Wiley and Cooper [5] described a (Na^+/K^+)-cotransport system that was inhibited by furosemide and other diuretics. Subsequently, these fluxes were shown to be dependent on external Cl [6,7]. Other cells and tissues possessing similar transport systems include Ehrlich ascites cells [2], duck red blood cells [8], mammalian kidney [9], flounder intestine [10] as well as the rabbit myometrium [11]. The role of the cotransport system in smooth muscle is uncertain. In Ehrlich ascites cells, Geck et al. [2] argued that the ouabain-inhibitable Na^+ pump and the furosemide-sensitive cotransport system worked antagonistically to regulate cell volume, the first acting against cell swelling and the second against cell shrinking. The mechanisms controlling cell volume in smooth muscle are unclear. Canine iliac arteries do not undergo any tissue swelling in the presence of ouabain [3] nor does Cl^- removal produce any marked changes in water contents (this study). At the present time, it is not possible to present persuasive arguments implicating the $(Na^+/K^+/Cl^-)$-cotransport system in regulation of cell volume in smooth muscle. Conversely, some of the experimental manipulations we have used (such as elevated concentrations of K^+, Rb^+ or removal of Na^+) could have altered cell volume which could have affected the transport processes studied. These possibilities have not been critically tested as yet.

Total Tl^+ uptake by canine iliac arteries can be subdivided into three components: a ouabain-inhibitable component that clearly represents transport on the K^+ limb of the classical Na^+/K^+ pump, a ouabain-insensitive but halide-sensitive component which may reflect the existence of a $(Na^+/K^+/Cl^-)$-cotransport process and a residual component, which may involve Tl^+-permeating biological membranes as a lipid permeable cation. In the latter context, it is instructive to note that Gutknecht [12] has recently noted that the permeability of Tl^+ across lipid bilayer membranes is very low and that lipid permeability may, in fact, in volve diffusion of Tl^+ as a complex with other inorganic and organic anions (such as Tl-Cl, Tl-NO_3). Although such complex formation cannot explain the characteristics of the halide-sensitive Tl^+ uptake noted here, it could explain residual Tl^+ uptake reasonably well.

Acknowledgements

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