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STIMULATION OF K^+ FLUXES BY DIURETIC DRUGS IN HUMAN RED CELLS

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Abstract—Two different families of diuretic drugs—(i) (aryloxy)acetic acid diuretics (ethacrynic acid, tienilic acid and (−)-indacrinone) and (ii) furopyridines [(±)-BN 50157 and (±)-cycletanide]—stimulate K^+ movements across human red cell membranes. The kinetic properties of this effect (K^+-specificity, saturability, optical isomerism, antagonism by structural analogues, etc.) strongly suggest that it is mediated by a K^+-transport system with a specific binding site for some diuretic drugs. The stimulated K^+ fluxes are resistant to ouabain, bumetanide and quinine, thus suggesting that they are not mediated by the Na^+,K^+-pump, Na^+,K^+-cotransport or by the Ca^{2+}-dependent K^+-permability ('Gardos effect'). The replacement of Cl^- by NO_3^- ions can either decrease, increase or have no effect on the stimulated K^+ fluxes, depending on the diuretic drug. Although not conclusive, these observations suggest that the K^+ fluxes are not mediated by stimulation of a chloride-dependent K^+ carrier. The study of structural analogues showed that the intensity of the stimulation of K^+ fluxes is strongly correlated with the magnitude of the natriuretic effect. Curiously, some antiallergic furopyridines are able to inhibit K^+ fluxes.

Several observations suggest that diuretic drugs may decrease renal Na^+ reabsorption by direct inhibition of a Na^+-transport carrier in different segments of the nephron (Fig. 1). The injection of ouabain directly into the renal artery inhibits basolateral Na^+-reabsorption which is catalysed by the Na^+,K^+-pump, inducing a profound natriuresis [1]. Furosemide and bumetanide provoke natriuresis by inhibition of another Na^+-transport carrier: the Na^+,K^+,Cl^--cotransport system which is located in the luminal side of Henle's loop cells [2, 3]. Amiloride inhibits a Na^+ carrier located apically in distal tubular cells [4]. Spironolactone antagonizes the aldosterone-induced synthesis of a Na^+-transport

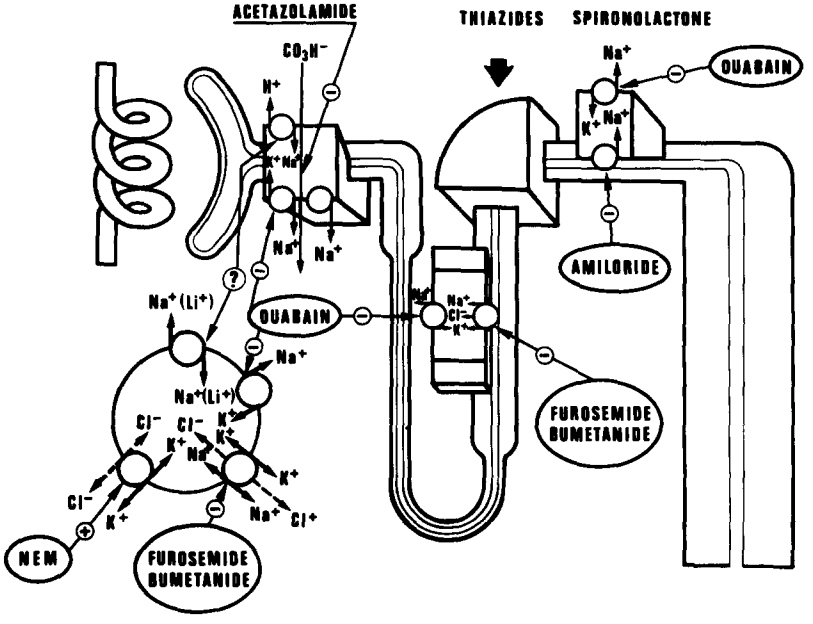


Fig. 1. Na^+- and K^+-transport systems in kidney and in human red cells (see text).

protein in distal and collecting tubules [4, 5]. Acetazolamide abolishes proximal tubular acidification, thus inhibiting the net reabsorption of $NaHCO_3$ [6]. The mechanism of action of thiazide drugs is unclear.

Direct action of diuretic drugs on Na^+-transport systems was further suggested by the inhibition of Na^+ movements across cell membranes of several cell types. Ouabain inhibits a Na^+,K^+-pump in most animal cells [7]. Furosemide and bumetanide inhibit a Na^+,K^+, Cl^--cotransport system in human [8], rat [9] and avian [3] erythrocytes, in vascular smooth muscle cells [10], in Ehrlich ascites tumour cells [11], etc. Amiloride inhibits apical Na^+ reabsorption in several epithelial tissues [12]. In addition, high doses of amiloride inhibit Na^+–H^+ exchange in the proximal tubule [13] and in striated muscle [14].

In contrast to the mode of inhibition by the drugs mentioned above tienilic acid, an (aryloxy)acetic acid diuretic [15], modifies K^+ rather than Na^+ fluxes in human red cells [16]. Tienilic acid-stimulated K^+ fluxes did not stem from the Na^+,K^+-pump, the Na^+,K^+-cotransport system or from a 'Gardos effect' (Ca^{2+}-dependent K^+-permeability) [16]. A similar effect could not be obtained with hydrochlorothiazide, amiloride or furosemide. In order to gain insight into the mechanism of action of natriuretic and antihypertensive drugs, we explored further whether other diuretics exhibit properties similar to tienilic acid, and which K^+-transport systems were affected.

MATERIALS AND METHODS

Na^+ and K^+ movements across human red cell membranes are mediated by several transport systems (Fig. 1 and refs. [7, 8, 16]). Na^+,K^+-Pump activity was considered as the ouabain-sensitive component of Na^+ and K^+ fluxes. The ouabain-resistant Na^+ and K^+ fluxes which are inhibited by loop diuretics (furosemide, bumetanide, etc.) correspond to the fluxes catalysed by the Na^+,K^+-cotransport system. The ouabain and bumetanide-resistant, Li^+-stimulated Na^+ efflux was equated to Na^+,Li^+-countertransport. Ouabain- and bumetanide-resistant Na^+ and K^+ effluxes in Mg^{2+}–sucrose medium were considered as the passive Na^+- and K^+-permeabilities (ground membrane cation leak).

Na^+ and K^+ fluxes catalysed by the Na^+,K^+-pump, Na^+,K^+-cotransport, Na^+,Li^+-countertransport, passive Na^+- and K^+-permeabilities and diuretic-stimulated K^+ fluxes were measured in fresh erythrocytes. All measurements were performed in duplicate or triplicate.

Preparation of red cells. Venous blood (20 ml) collected in heparinized tubes was centrifuged at 1750 *g* for 10 min, and the plasma and buffy coat were aspirated. The red cell pellet was used immediately or stored at 4° for no more than 2 days in a preserving solution containing (mM): 140 KCl, 10 NaCl, 1 $MgCl_2$ and 2.5 Na^+ phosphate (pH 7.2 at 4°).

Simultaneous measurement of Na^+,K^+-pump, Na^+,K^+-cotransport, Na^+,Li^+-countertransport and oaubain- and bumetanide-resistant Na^+ and K^+ fluxes in human red cells. Red cells were washed five times with cold 110 mM $MgCl_2$ and resuspended to a haem-

Table 1. The effect of diuretic drugs on Na^+ and K^+ transport in fresh human erythrocytes

Condition	Na^+,K^+-pump	Na^+,K^+-Cotransport system		Na^+,Li^+-counter-transport	Ouabain and bumetanide-resistant	
	Ouabain-sensitive Na^+ efflux	Bumetanide-sensitive Na^+ efflux	Bumetanide-sensitive K^+ efflux	Li^+-stimulated Na^+ efflux	Na^+ efflux	K^+ efflux
Control	1630 ± 210 (8)	160 ± 80 (8)	180 ± 90 (8)	95 ± 25 (6)	110 ± 40 (7)	895 ± 80 (7)
Tienilic acid, 1 mM	1370 ± 260 (6)	100 ± 40 (6)	120 ± 40 (6)	99 ± 41 (3)	145 ± 50 (6)	1445 ± 110 (5)
Ethacrynic acid, 0.4 mM	1490 ± 260 (4)	0 ± 10 (4)	*	69 ± 21 (4)	190 ± 60 (5)	3590 ± 500 (5)
(±)-Indacrinone, 0.7 mM	1310 ± 330 (3)	80 ± 30 (5)	80 ± 50 (5)	71 ± 38 (3)	125 ± 30 (5)	1305 ± 95 (5)
(±)-Cycletanide, 0.85 mM	1640 ± 220 (5)	150 ± 80 (4)	200 ± 60 (4)	95 ± 45 (3)	130 ± 60 (4)	1215 ± 85 (4)
(±)-BN 50157, 0.6 mM	1450 ± 300 (3)	130 ± 30 (4)	*	108 ± 46 (4)	230 ± 85 (5)	5070 ± 560 (5)

Fluxes are expressed in μmol (l. cells × hr)$^{-1}$. Values are given as mean ±S.D. The number of experiments is given in parentheses.
* The bumetanide-sensitive K^+ efflux is only a small fraction of the total K^+ efflux. Thus its determination has a high experimental error.

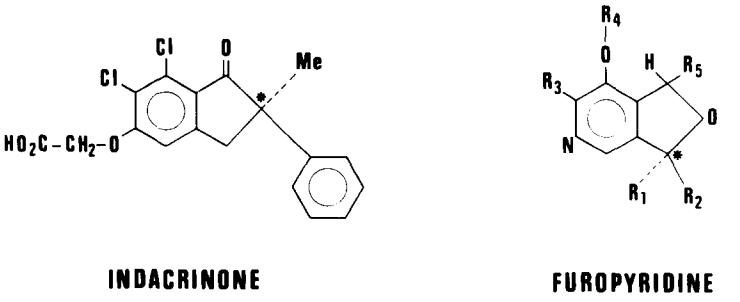


Fig. 2. Structural analogy between indacrinone and furopyridines [(±)-cycletanide, (±)-BN 50157, etc.].

atocrit of 20–25% in Mg^{2+}–sucrose medium. The Mg^{2+}–sucrose medium contained (mM): 75 $MgCl_2$, 85 sucrose, 10 MOPS–Tris (pH 7.4 at 37°) and 10 glucose.

Human red cell suspensions were added in the cold (final haematocrit 4–5%) to different solutions containing buffered Mg^{2+}–sucrose medium as a basic constituent plus the following additions (mM): (1) 2 KCl, (2) 0.1 ouabain, (3) 0.1 ouabain plus 0.02 bumetanide and (4) 10 LiCl, 0.02 bumetanide and 0.1 ouabain. Osmolarities were maintained at 295 ± 10 mOsM. At $t = 0$, the tubes were transferred to a 37° water bath for further incubation. External Na^+ and K^+ concentrations were measured in the supernatants at time (min) 0 (media 3 and 4), 30 (medium 1), 60 (medium 4) and 120 (media 2 and 3), after which the cell suspensions were transferred to the cold and spun down for 4 min at 1750 *g* at 4°.

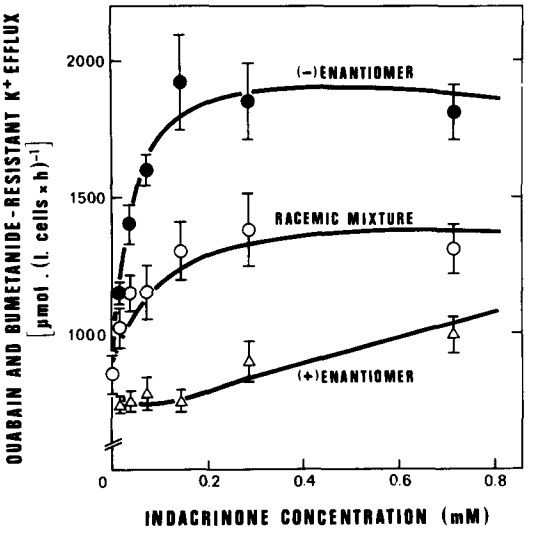


Fig. 3. Optical isomerism of the indacrinone-stimulated K^+ efflux. The (−)-enantiomer, but not the (+)-enantiomer, is able to stimulate K^+ fluxes in human red cells. This effect is correlated with the higher diuretic potency of the (−)-enantiomer. A slight but significant inhibition of K^+ efflux is observed with the (+)-enantiomer. Values in this figure represent mean ±S.D. of four experiments.

Na^+ and K^+ effluxes were computed using the following equation:

$$\text{Cation efflux} = \frac{(D_{cat}) \times (1 - \text{final haematocrit})}{(\text{final haematocrit}) \times 0.85}$$

where 0.85 is the correction factor for the Na^+ and K^+ reading of the Eppendorf flame photometer; D_{cat} (μmole/l. supernatant) is the difference between the external cation concentration (Na^+ or K^+) after incubation at 37° and that at zero time. The Na^+,K^+-pump activity was calculated by subtracting Na^+ efflux in the presence (medium 2) from that in the absence of ouabain (medium 1). The Na^+,K^+-co-transport fluxes were obtained by subtracting Na^+ and K^+ effluxes in the presence of ouabain plus bumetanide (medium 3) from those in the presence of ouabain alone (medium 2), while for calculation of Na^+, Li^+-countertransport, Na^+ efflux in medium 3 was subtracted from that in medium 4. The Na^+ and K^+ effluxes in medium 3 were taken as the 'ouabain- and bumetanide-resistant Na^+ and K^+ fluxes'.

Haemoglobin absorbance in the supernatant was very slight and did not vary as a function of time, thus indicating no K^+ release due to cell lysis during the flux experiment.

The effect of drugs and Cl^- *substitution.* To study the effect of several diuretic drugs on Na^+ and K^+ transport in human red cells, the compounds were added from freshly prepared, concentrated stock solutions in water, ethanol or dimethyl sulphoxide. In experiments with Cl^- substitution, ouabain- and bumetanide-resistant K^+ efflux was studied in Cl^- and NO_3^- media using a protocol similar to that described above. Fresh erythrocytes were washed three times with cold 110 mM $Mg(NO_3)_2$ and resuspended at a haematocrit of 20–25% in NO_3^- medium containing (mM): 75 $Mg(NO_3)_2$, 85 sucrose, 10 MOPS–Tris (pH 7.4 at 37°) and 10 glucose, 0.1 ouabain and 0.02 bumetanide. Aliquots of cell suspensions were added at 4° (final haematocrit of 4–5%) to NO_3^- and Mg^{2+}–sucrose media. The tubes were then incubated at 37°. External K^+ concentration was measured at 0 and 30 or 60 min intervals. Ouabain and bumetanide-resistant K^+ effluxes were computed as described above.

Drugs. The drugs were provided by Anphar Laboratories (tienilic acid), Leo Laboratories

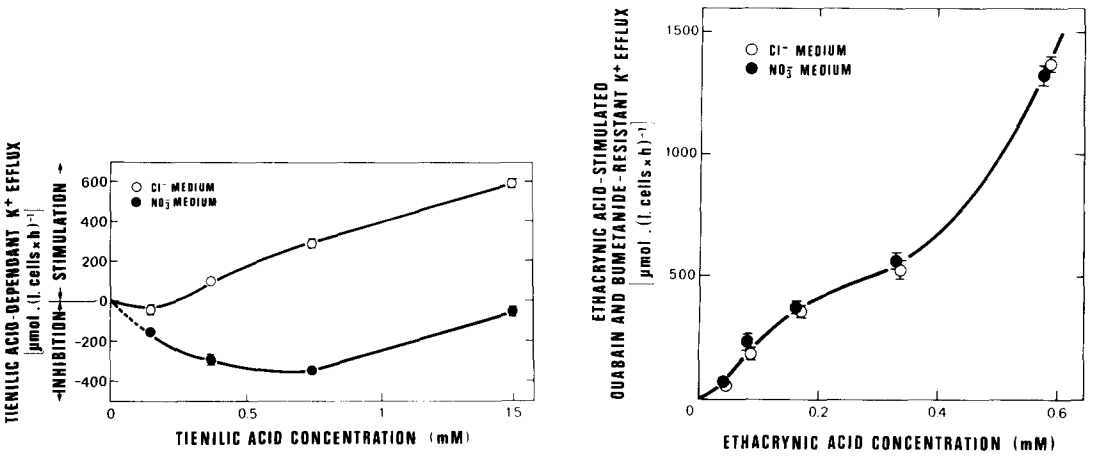


Fig. 4. (a) The effect of anion replacement on the stimulation of K^+ fluxes by tienilic acid. Tienilic acid stimulates K^+ fluxes in Cl^- medium and inhibits K^+ fluxes in NO_3^- medium. Values are given as mean ± range. (b) Stimulation of K^+ fluxes by ethacrynic acid in Cl^- and NO_3^- media. Values are given as mean ± range.

(bumetanide), I.H.B. Research Laboratory (cycletanide and other furopyridines) and Merck, Sharp & Dohme–Chibret Laboratories (France) (indacrinone and ethacrynic acid).

RESULTS

Stimulation of ouabain- and bumetanide-resistant K^+ fluxes by diuretic drugs

In a screening of diuretic drugs on Na^+ and K^+ transport across human red cell membranes, we found previously that tienilic acid stimulates ouabain- and bumetanide-resistant K^+ fluxes (ref. [16] and Table 1). Here we have investigated whether other diuretic drugs share similar properties.

Table 1 shows that, in addition to tienilic acid, (±)-indacrinone, ethacrynic acid, (±)-BN 50157 and (±)-cycletanide are also able to stimulate K^+ movements across red cell membranes. It is interesting to note that some of these drugs, particularly ethacrynic acid, are also able to inhibit the fluxes catalysed by the Na^+,K^+-cotransport system (Table 1).

Stimulation of K^+ fluxes by (±)-indacrinone and optical isomers

The (±)-indacrinone molecule contains one asymmetric carbon (Fig. 2). Interestingly, the (−)-enantiomer shows a much more pronounced diuretic effect than the (+)-enantiomer [17]. This property allowed us to investigate whether stimulation of K^+ fluxes correlates with the diuretic effect.

Figure 3 shows that (−)-indacrinone strongly stimulates ouabain- and bumetanide-resistant K^+ fluxes in human erythrocytes. Conversely, (+)-indacrinone showed a very weak inhibitory effect, and the racemic mixture showed about half the activity of the (−)-enantiomer.

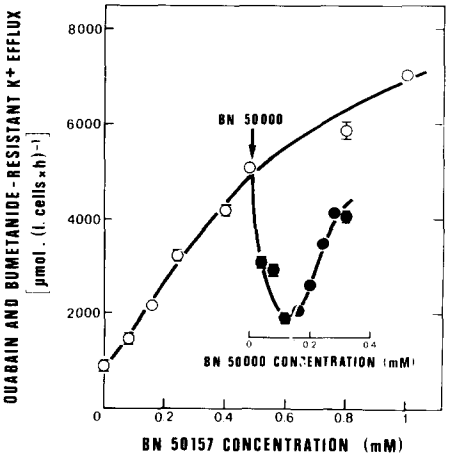


Fig. 5. Stimulation of K^+ fluxes in human erythrocytes by (±)-BN 50157. Antagonist effect of (±)-BN 50000. Values are given as mean ± range.

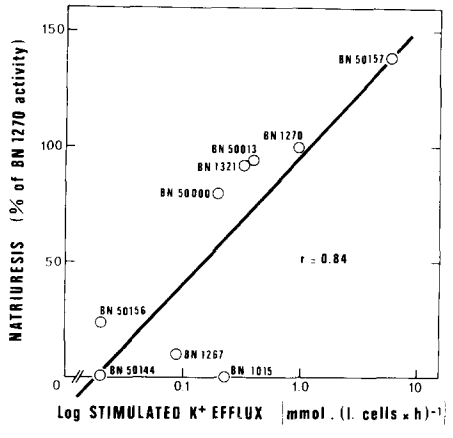


Fig. 6. Correlation between stimulation of K^+ fluxes in erythrocytes and natriuretic activity for furopyridines. The natriuretic activity is indicated as % of the (±)-cycletanide (BN 1270) activity. The experimental errors are not indicated (see Table 3). The amino or F-derivatives have been excluded from this figure.

Table 2. The effect of anion replacement on the stimulation of K^+ fluxes by diuretic drugs in human red cells

Drug	Cl^- medium		NO_3^- medium	
	K^+ efflux [μmol (l. cells × hr)$^{-1}$]	ΔK^+ efflux [μmol (l. cells × hr)$^{-1}$]	K^+ efflux [μmol (l. cells × hr)$^{-1}$]	ΔK^+ efflux [μmol (l. cells × hr)$^{-1}$]
Control	1040 ± 130 (14)		1660 ± 180 (14)	
Tienilic acid, 1 mM	1530 ± 200 (6)	480 ± 140 (6)	1460 ± 200 (6)	−180 ± 120 (6)
Ethacrynic acid, 0.7 mM	3620 ± 800 (7)	2550 ± 850 (7)	4480 ± 650 (7)	2760 ± 620 (7)
(±)-Cycletanide, 1 mM	1990 ± 190 (8)	980 ± 260 (8)	2260 ± 390 (8)	620 ± 270 (8)
(±)-BN 50157, 0.4 mM	2540 ± 220 (4)	1510 ± 250 (4)	4100 ± 550 (4)	2450 ± 750 (4)
(−)-Indacrinone, 0.4 mM	1920 ± 200 (4)	930 ± 210 (4)	3140 ± 250 (4)	1500 ± 300 (4)

The values in this table are given as mean ± S.D. The number of experiments is indicated in parentheses.

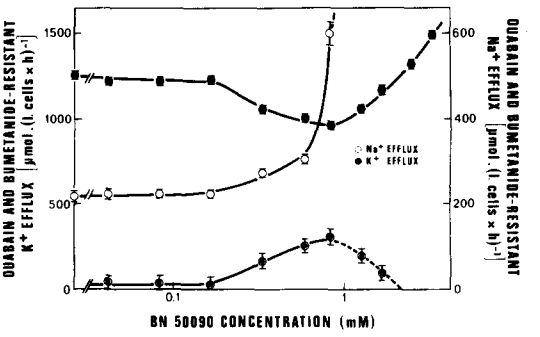


Fig. 7. Inhibition of K^+ efflux in NO_3^- medium by (±)-BN 50090. This effect is specific for K^+ ions. The inhibited K^+ efflux is indicated by ⊙. Values are given as mean ± range.

The effect of chloride replacement

The stimulation of ouabain- and bumetanide-resistant K^+ fluxes by diuretic drugs was measured in both Cl^- and NO_3^- media. Some important aspects of this kind of experiment deserve a preliminary comment. First, the ouabain- and bumetanide-resistant K^+ efflux is much higher in NO_3^- than in Cl^- medium (Table 2). In addition, the K^+ efflux in Cl^- medium depends on the previous treatment of the cells. Indeed, it is higher in cells washed in NO_3^- medium than in cells washed in Cl^- medium (compare the control values of K^+ efflux of Table 1 with those of Table 2). These experimental details may introduce some artefacts in this kind of experiment.

Taking into account the above considerations, Table 2 and Figs. 4a and 4b, show that: (i) the presence of Cl^- is required in order to obtain stimulation of K^+ fluxes by tienilic acid (a significant inhibition was observed in NO_3^- medium); (ii) (±)-cycletanide stimulates more in Cl^- than in NO_3^-

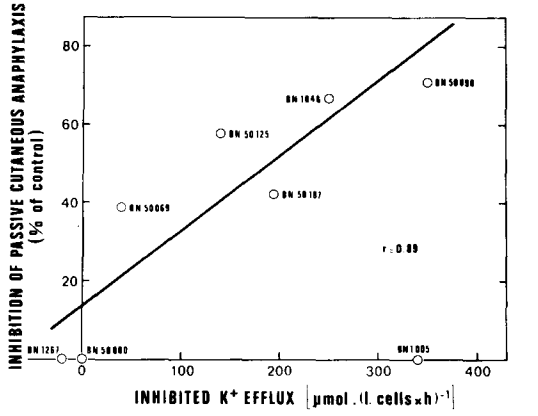
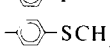


Fig. 8. Correlation between antiallergic activity and inhibition of K^+ fluxes in NO_3^- medium for (±)-BN 50090 analogues. Almost all of these analogues are amino or F-furopyridines (see Table 3). If BN 1005, which is neither an amino nor an F-derivative, is excluded, a good correlation is found. The antiallergic activity was measured as the inhibition of passive cutaneous anaphylaxis in rats (vascular leakage of Evans blue into a cutaneous pupula).

Table 3. The effect of furopyridines on K^+ fluxes in human erythrocytes

(±)-BN product	Radical substituents*					Natriuretic activity in rats, % of BN 1270	% Inhibition of passive cutaneous anaphylaxis in rats†	Effect on erythrocyte K^+ efflux‡ [mmol (l. cells × hr)$^{-1}$]	
	R^1	R^2	R^3	R^4	R^5			Cl medium	NO_3^- medium
1270 (cycletanide)	H		$-CH_3$	H	H	100	34	980 ± 260 (8)	620 ± 270 (8)
50000	H		$-CH_3$	H	H	80	0	190 ± 50 (5)	0 ± 10 (5)
1048	H		$-CH_3$	H	H	305	67	−180 ± 70 (6)	−250 ± 70 (6)
1005	H		$-CH_3$	H	H	81	0	−110 ± 60 (6)	−340 ± 90 (6)
1267	CH_3		$-CH_3$	H	H	10	0	90 ± 70 (5)	10 ± 40 (5)
50125	H		$-CH_2NH_2$	H	H	11	58	−70 ± 40 (5)	−140 ± 50 (5)
50090	H		$-CH_2NHCH_3$	H	H	—	71	−140 ± 80 (6)	−350 ± 90 (6)
50069	H		$-CH_2N(CH_3)_2$	H	H	25	39	−135 ± 30 (5)	−40 ± 30 (5)
50156	H		$-CHOH-CH=CH_2$ $\lvert$ $CH_2\ N(CH_3)_2$	H	H	24		20 ± 70 (4)	35 ± 50 (4)
50157	H		$-CH=CH_2$	H	H	139	25	6070 ± 920 (5)	8680 ± 1700 (5)
50187	H		$-CH=CH_2$	H	H		42	−73 ± 40 (3)	−195 ± 120 (3)
1015	H		$-CH_3$	$-CH_2-CO_2H$	H	—	0	236 ± 70 (4)	82 ± 55 (4)
1321	H		$-CH_3$	glycopyranose	H	92		325 ± 25 (4)	50 ± 40 (4)
50144	H	H	$-CH_3$	H		—		20 ± 30 (3)	0 ± 10 (3)
50013	H—CH=CH—		$-CH_3$	$-CH-CO_2H$ $\lvert$ CH_3	H	94	0	395 ± 35 (5)	145 ± 40 (5)

* See formula in Fig. 2.
† Inhibition of the vascular leakage of Evans Blue into a cutaneous papula.
‡ Values are given as mean ± S.D. The number of experiments is indicated in parentheses. Drugs were assayed at 1 mM concentration.

medium; (iii) ethacrynic acid stimulates to the same extent in Cl^- and NO_3^- media; and (iv) (−)-indacrinone and (±)-BN 50157 stimulate more in NO_3^- than in Cl^- medium.

The above results show that, with the exception of tienilic acid, the stimulation of K^+ fluxes by diuretic drugs is not entirely dependent on the presence of Cl^- ions.

Stimulation of K^+ fluxes by furopyridine diuretics

Among the diuretics studied, a furopyridine, (±)-BN 50157 (see Table 3), is the most potent activator of erythrocyte K^+ fluxes which we have found. A dose–response curve showed that the effect may be observed for doses as low as 0.1–0.2 mM, reaching a stimulation of 5000–7000 μmole (l. cells × hr)$^{-1}$ at 1 mM concentration (Fig. 5). Interestingly, the effect of (±)-BN 50157 may be antagonized by other furopyridines such as (±)-BN 50000 (Fig. 5). This result further suggests that the stimulated K^+ fluxes are catalysed by a K^+-transport system with a specific binding site for some diuretic drugs.

A possible correlation between the stimulation of K^+ fluxes and the natriuretic effect was investigated further with several furopyridines. Surprisingly, not all diuretic furopyridines stimulate K^+ fluxes (Table 3). Indeed, some furopyridines are able to inhibit K^+ fluxes, particularly in NO_3^- medium (Table 3 and Fig. 7). Most of these inhibitory compounds have an amino or F group and are characterized by showing antiallergic properties (Table 3). If only the former group of furopyridines is taken into consideration, a good correlation may be found between the stimulation of K^+ fluxes and the natriuretic activity (Fig. 6). For the inhibitory furopyridines, a good correlation was found between the inhibition of K^+ fluxes and the antiallergic activity (Fig. 8).

A kinetic analysis of the stimulation of erythrocyte K^+ fluxes by furopyridine diuretics

The stimulation of erythrocyte K^+ fluxes by furopyridine [and also by (aryloxy) acetic acid] diuretics does not follow simple Michaelis–Menten kinetics. For instance, a Hanes plot (see ref. [18]) of the stimulation of K^+ fluxes by (±)-BN 50157 only fits a straight line by a model with more than one receptor site per transport unit (Fig. 9a). This illustrates the kinetic complexity of the phenomenon and hampers an adequate demonstration of antagonism between different furopyridines for the same receptor site. Nevertheless, Fig. 9b suggests that (±)-BN 50157 and (±)-BN 50000 may compete for two receptor sites in the same transport molecule.

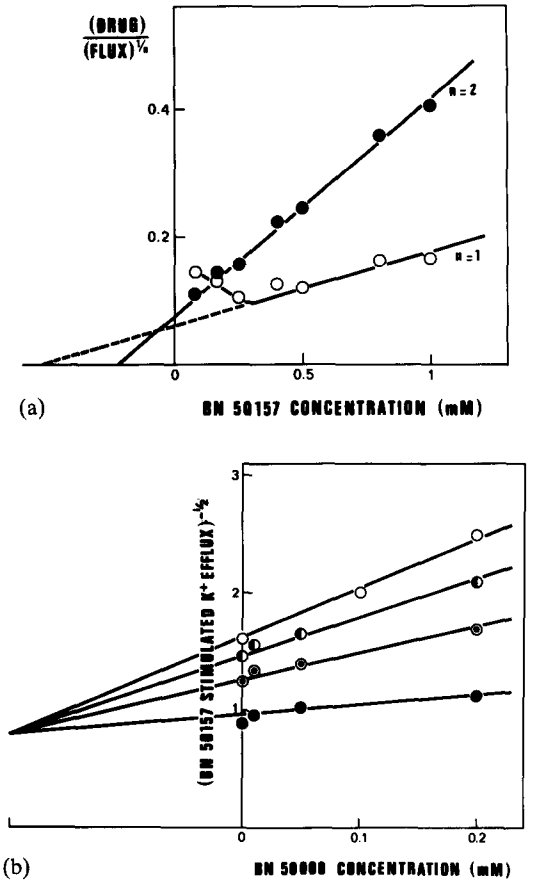


Fig. 9. (a) Hanes plot of the (±)-BN 50157-stimulated K^+ efflux (see ref. [18] for kinetic aspects). n indicates the number of binding sites per transport unit. The experimental values fit a straight line for $n = 2$, whereas for $n = 1$ the points at low diuretic concentrations curve upwards, deviating from a straight line. (b) (±)-BN 50157-stimulated K^+ efflux as a function of (±)-BN 50000 concentration. Each curve represents the experimental points at a constant (±)-BN 50157 concentration assuming two binding sites per transport unit. The same intercept for the four straight lines suggests competition between the two furopyridines for the same binding sites.

DISCUSSION

The main result of this paper is that two different families of diuretic drugs—(i) (aryloxy)acetic acid diuretics (ethacrynic acid, tienilic acid and (−)-indacrinone) and (ii) furopyridines ((±)-BN 50157 and (±)-cycletanide)—are able to stimulate K^+ movements across human red cell membranes. The study of structural analogues showed that the magnitude of this effect correlates with the intensity of the natriuretic effect.

The diuretic-stimulated K^+ flux is characterized by the following properties: (i) a quasi-specificity for K^+ ions [16]; (ii) saturability with an increase in internal K^+ concentration [16]; (iii) differential stimulation by the optical isomers of (±)-indacrinone; and (iv) inhibition by some structural analogues of (±)-BN 50157 such as (±)-BN 50000. These properties strongly suggest that the above diuretics are able to stimulate a K^+-transport system by interacting with one or more specific binding sites. The K^+ fluxes catalysed by this transport system are resistant to ouabain, furosemide, bumetanide, quinine, carbocyanin and EGTA (see this paper and ref. [16]) thus suggesting that they are not mediated by the Na^+,K^+-pump, Na^+,K^+,Cl^--cotransport system or by the Ca^{2+}-dependent K^+-permeability ('Gardos effect').

Lauf has recently found a new K^+-transport system in erythrocytes from a marine teleost [19] and LK

sheep [20]. The K^+ translocation by this carrier requires the presence of Cl^- and may be stimulated by *N*-ethylmaleimide (NEM) or by a hypotonic environment. The stimulation of this transport system induces a net extrusion of KCl and cell shrinking, thus suggesting that it is involved in the regulation of cell volume [19, 20].

We have recently observed that in human erythrocytes NEM stimulates a Cl^--dependent K^+-carrier [21]. We thus decided to investigate whether furopyridines and (aryloxy)acetic acid diuretics can also stimulate this carrier.

We have found that, depending on the diuretic drug, the K^+-flux stimulation may be higher, equal or lower in Cl^- than in NO_3^- medium. Although not conclusive, these results suggest that NEM and diuretic drugs stimulate two *different* K^+-transport systems.

Our results clearly show that the natriuretic effect of some diuretic drugs is correlated with the stimulation of a K^+-carrier mechanism. This opposes the classical view which assumes that diuretic drugs *inhibit* one or more Na^+-transport systems involved in renal Na^+ reabsorption (however, some of these 'K^+-stimulating diuretics', particularly ethacrynic acid, may also inhibit the Na^+,K^+,Cl^--cotransport system and thus renal Na^+ reabsorption). In several kinds of cells, such as vascular smooth muscle cells, noradrenergic neurons and kidney cells, the membrane potential is strongly dependent on K^+ permeability. On the other hand, a change in membrane potential in some target cells modulates the secretion of natriuretic agents such as prostacyclin [22]. This mechanism of action of (aryloxy)acetic acid and furopyridine diuretics deserves further investigation.

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