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The Mechanism of Action of Amipramizide*

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Summary. Amipramizide is capable of inhibiting active sodium transport by isolated bladder, colon and skin of the toad, *Bufo marinus*, when added in small amounts ($\leqslant 10^{-5}$ M) to the solution coming in contact with the outer surface of the preparations. Inhibition occurs rapidly, is proportionate to dose and is reversible. No significant effect results from the presence of large amounts of the drug in the solution bathing the inner surface of the membranes.

From measurements of "active sodium transport pool" in toad bladder tissue, it appears that pool size was decreased in proportion to sodium transport; on the other hand, amphotericin B eliminated the action of amipramizide on sodium transport. Therefore amipramizide is assumed to act by impeding the combination of sodium ions with carriers ("permeases") whose presence is postulated at the apical border of these biological membranes.

Amipramizide did not prevent the preparations from reacting to aldosterone, insulin or vasopressin by increased sodium transport provided the latter had not been suppressed by the drug. With respect to the permeability of toad bladder to water, it was not modified by this drug either.

Amipramizide could be recovered chemically unaltered from incubation medium and from tissue at the end of the experiments.

Key-Words: Amipramizide — Toad Epithelia — Active Sodium Transport — Hydrodynamic Flow — Hormones.

Among the drugs having the capacity to interfere with tubular sodium reabsorption, amipramizide, or MK-870 [5,17], seems potentially useful as a therapeutic agent: although its natriuretic effect is modest, it has an appreciable potassium-sparing action which can diminish or offset the kaliuresis induced by more potent diuretics [1,3,6,7,24,26,28,30, 35,38,39,41,43,44]. The excretion of hydrogen ions is also reduced following the administration of amipramizide [3,7,25,26,28,44]. With respect to all these actions amipramizide is similar to triamterene and the spirolactones, and like them it is understood to exert its effect upon the distal part(s) of the nephron [3]. On the other hand amipramizide

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differs from the spirolactones since it is effective in the absence of aldosterone [23]—like triamterene [34] to which, however, amipramizide is chemically unrelated.

EIGLER, KELTER, and RENNER [20] have recently shown that active sodium transport by frog skin was inhibited by low concentrations of amipramizide, only when the outward-facing surface of the preparation was exposed to the drug. Furthermore, sodium transport was found by these investigators to be immediately restored following removal of amipramizide.

Such characteristics prompted the studies reported here, which were aimed at elucidating the mechanism whereby amipramizide interferes with active sodium transport, in order to arrive at a better understanding of its mode of action as a diuretic.

Material and Methods

Toads, *Bufo marinus*, kept on moist peat and fed once weekly, were transferred to shallow containers in which they were maintained half-immersed in tap water for a few days before use. After pithing, ventral skin, colon and urinary bladder were dissected and incubated as devised by USSING and ZERAHN [42]. Short-circuit current and transmembrane electrical potential were measured manually at 5—15 minute intervals. In some instances, the mucosa only of toad colon was used after stripping off of underlying muscularis and serosa. Incubated surface was generally 2 cm^2 in the case of colon, 3.14 cm^2 for bladder and skin.

Incubation fluid was usually Ringer's, containing (in mM/ml): NaCl, 115.0; $KHCO_3$, 2.5; $CaCl_2$, 1.0. In some cases Ringer's composition was modified by replacement of Cl^- with $SO_4^=$, in other instances Na^+ was replaced with Mg^{++}; care was always taken to keep osmolality of these solutions at 0.225 by addition of sucrose.

A stock solution of amipramizide hydrochloride dihydrate (MW 302), 0.15 mg/ml, was prepared and added in variable amounts to the solution used for incubation. For most experiments, the preparations were exposed only to amipramizide; removal of the latter was accomplished by draining the drug-containing fluid and appropriate rinsing; incubation was then resumed with drug-free medium.

Measurement of the "active sodium transport pool" in the toad bladder was carried out as described previously [15]. In another set of experiments, bidirectional sodium fluxes were measured simultaneously, by means of ^{24}Na and ^{22}Na, across toad bladder [32, 41] in order to assess equivalence between net sodium flux and short-circuit current during exposure to amipramizide. Sometimes only the sodium fluxes inward were measured with radiosodium, and compared with short-circuit current.

The ionic composition of toad bladder tissue was determined by flame photometry performed on 0.1 M HNO_3 eluates of the dry residue, after estimation of tissue water by weighing before and after standing overnight at 95° C.

Fixation of amipramizide onto toad bladder tissue was evaluated by comparing the volumes of distribution of inulin and amipramizide expressed as percent tissue water, after a 1 hour incubation period. Both substances were added simultaneously to the solution on the outside of the membranes — the concentration of inulin in this solution was 1.0 μc/ml as ^3H-methoxy inulin (S.A.: 64 μC/mg); that of amipramizide, labelled with ^{14}C in the guanidine ring (S.A.: 1.0 $\mu C/\mu M$), was

0.15 μC/ml. At the end of incubation, the solution containing radioactive material was sampled for liquid scintillation spectrometry; so were the eluates of the tissue, prepared as described above.

The possibility of amipramizide undergoing chemical transformation after contact with toad bladder, was evaluated by comparing R_f properties of the radioactive compound to those of pure substance, when chromatographed on paper according to BAER, JONES, SPITZER and RUSSO [3].

A series of experiments was undertaken to evaluate possible changes in permeability to water of toad bladder exposed to amipramizide. Incubation was carried out according to BENTLEY [4], Ringer's diluted to 20% with water being used inside; one bladder half served as a control while the matched preparation, from the same toad, was treated with the drug, 5×10^{-6} M, added to the solution inside.

Insulin (ox insulin Novo, 10 times recrystallized, 40 U/ml), aldosterone (Aldocorten CIBA, 0.5 mg/ml) and lysine-vasopressin (Octapressin Sandoz, 5 U/ml) were added, when used, to the serosa-bathing compartment. Final concentrations achieved were 0.125 U/ml, 5×10^{-6} M and 0.1 U/ml, respectively; for the permeability experiments meant to measure osmotically induced water flow across toad bladder, the concentration of vasopressin in the incubation fluid was 25 μU/ml.

Results

1. Depression by Amipramizide of Electrical Activity of Toad Epithelia

a) When small amounts of amipramizide were added to the Ringer's fluid bathing the outside surface of each of the types of membranes employed in the present study, short-circuit current and transmembrane potential dropped within minutes to a lower level of activity. The term "outside" designates the cornified epithelial surface of the skin, and the luminal (or mucosal) surface of the colon or of the bladder.

As seen on Fig. 1 illustrating the behaviour of toad bladder, the action of the drug occurred very quickly, and seemingly left the membranes unharmed as evidenced by prompt and complete recovery following removal of the drug.

Electrical potential differences were influenced almost to the same extent as short-circuit current. For the membranes whose data are presented on Fig. 1, the current averaged 18.7 μ Amp/cm² in the absence of amipramizide and it dropped to a mean value of 10.5 μ Amp/cm² during exposure to the drug; the transmembrane potential in turn dropped from a mean of 16.2 mV to a mean of 10.6 mV, in the presence of the drug.

Toad skin behaved similarly, as noted in Fig. 2; with toad colon, the only difference is a quantitative one: the amounts of amipramizide required to depress sodium transport 50% or more across this preparation were at least one order of magnitude larger than with bladder and skin (Fig. 2).

b) The effect of amipramizide on the electrical activity of toad epithelia was proportional to the concentration of the drug on the outside,

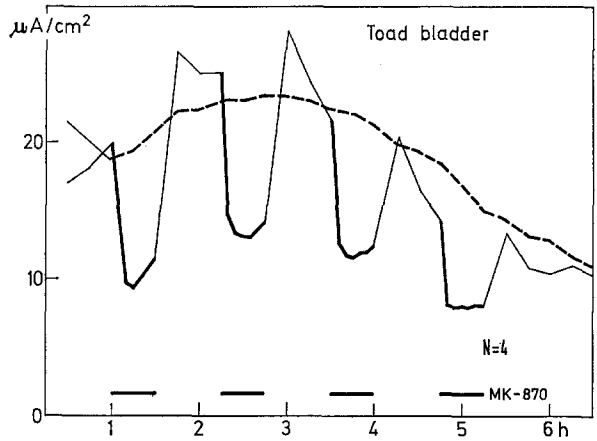

Fig. 1. Inhibitory effect of amipramizide (MK-870) on short-circuit current across isolated toad bladder. In 4 instances, one bladder half served as control (– – –) while the matched preparation (——) was exposed repeatedly to 4×10^{-7} M MK-870 added to the outside for periods lasting 30 minutes each. Removal of the drug resulted in short-lived rebounds of activity; the latter was approximately the same for treated and control membranes at the end of incubation

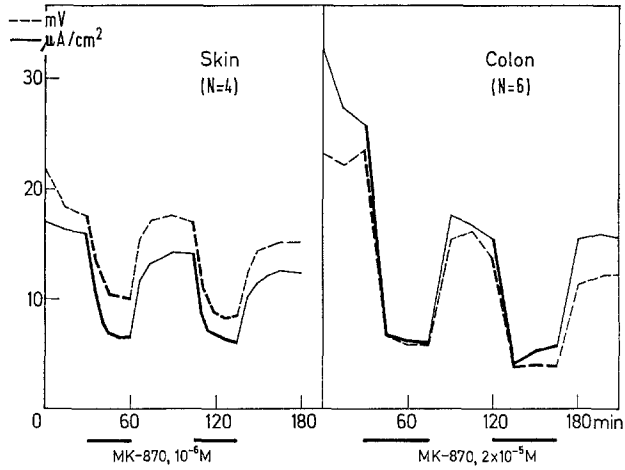

Fig. 2. Inhibition of electrical activity of toad skin and colon exposed to amipramizide (MK-870). Both preparations behaved similarly when their outside-facing surfaces were in contact with MK-870; with toad colon, a larger concentration of the drug was required for bringing about the same depression of sodium transporting activity. Note parallelism between variations of short-circuit current and those of transmembrane potential

as appears from Fig. 3; again, there was complete recovery of the preparations chosen, irrespective of the amplitude of inhibition achieved.

The pattern was analogous for toad colon, since 75% inhibition resulted from exposure to 4×10^{-5} M amipramizide while there was a 50% drop when 10 times less drug was used ($N = 6$).

c) The reaction of these epithelia to amipramizide is reminiscent of the depression of sodium transport which occurs when they are incubated with their outside surface exposed to solutions containing little sodium

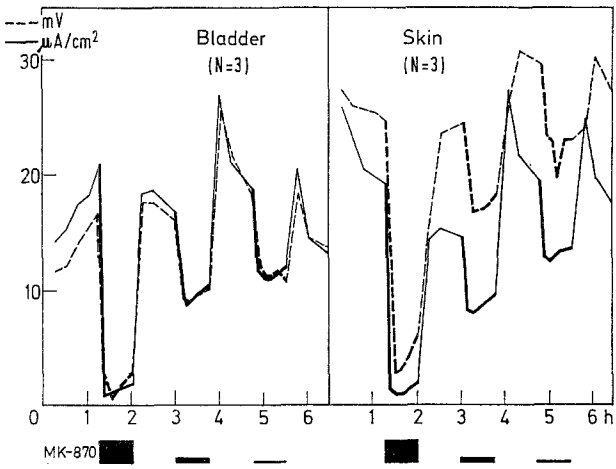

Fig. 3. Proportionality between the concentration of amipramizide (MK-870) in incubation fluid and the amplitude of the drug-induced inhibition of electrical activity of toad bladder and skin. Doses used were 4×10^{-6} M first, then 10^{-6} M, and finally 4×10^{-7} M. Again, note similitude of variations in short-circuit current and transmembrane potential

[9,22]. Therefore, both manipulations were attempted in sequence on bladder and skin; from Fig.4, it is obvious that the reaction of these membranes was indeed identical, at least qualitatively.

d) Amipramizide acted as inhibitor of sodium transport only when added to the outside-bathing solution. Thus, when toad bladder and colon were exposed for 2 hours to large amounts of the drug added on the inside, the activity of the treated preparations did not differ appreciably from that of matched controls, as noted from Fig.5; furthermore, under the influence of hormones which are capable to induce a brisk increase in short-circuit current across these preparations—vasopressin in the case of bladder [32], insulin in the case of stripped colon [14]—the stimulation was as pronounced in the presence as in the absence of amipramizide.

Even after overnight exposure of the inner, serosal surface of toad skin to the drug, 2×10^{-5} M, a stimulation of short-circuit current could still be elicited as a result of addition of antidiuretic hormone.

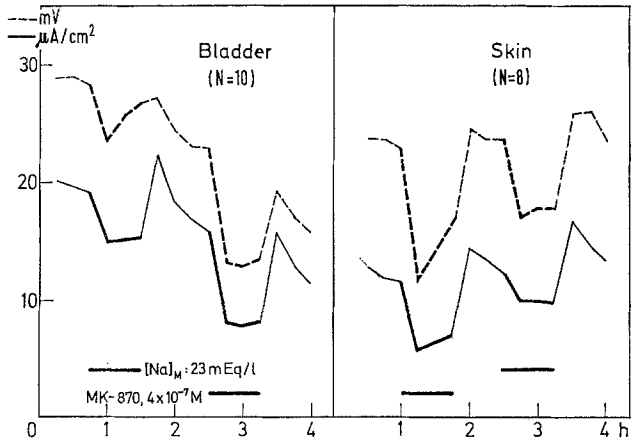

Fig. 4. Decreased electrical activity of toad bladder and skin whose outside-facing surfaces were exposed either to amipramizide (MK-870) or to low sodium Ringer's. Note existence of rebound in short-circuit current after removal of modified incubation fluid. Extent of dilution of sodium, by appropriate mixing of Ringer's with sodium-free Ringer's, was determined by flame photometry in each instance; sodium concentration averaged 23 mEq/l, one fifth of that in unmodified Ringer's

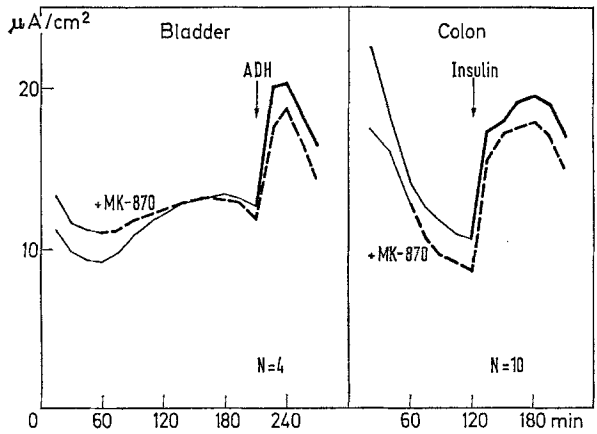

Fig. 5. Lack of influence of amipramizide (MK-870) added in large amounts to the serosal (inner) side of toad bladder and colon. These preparations were incubated in pairs, one half serving as a control for the matched piece exposed (after 60 minutes of incubation) to MK-870, 2×10^{-5} M. This treatment failed to influence significantly short-circuit current. The reaction of bladder to vasopressin (ADH), or of stripped colon to insulin, was likewise unaffected. Colon mucosa had to be dissected free from underlying layers in order to react to insulin *in vitro* [14]

The mean currents ($\mu A/cm^2 \pm$ S.E.) were 13.2 ± 2.5 for the control preparations, and 15.3 ± 2.5 for those exposed to amipramizide for

$\pm$ 15 hours ($N = 5$ pairs); during the second half-hour that followed introduction of this hormone into the incubation fluid, currents had risen to 23.3 $\pm$ 2.5 and 22.4 $\pm$ 2.8, respectively.

2. Equivalence between Net Sodium Flux and Short-Circuit Current during Exposure of Toad Epithelia to Amipramizide

From simultaneous measurement of radiosodium fluxes in both directions across 4 toad bladder whose activity had been depressed by amipramizide, 4×10^{-7} M, it appears that short-circuit current remained a reliable expression of net, active sodium transport under such circumstances (Table 1). Before addition of the drug, the sodium transporting activity of the preparations, computed from short-circuit current, had been much higher since it averaged 4.945 μEq/hour.

Table 1. *Bidirectional sodium flux measurements on toad bladder treated with amipramizide (Means $\pm$ S.E.)*[a]

Short-circuit current (μ Eq Na/hour) (1)	Unidirectional sodium flux (μ Eq Na/hour)		Ratio $\frac{(2)-(3)}{(1)} \times 100$
	M → S (2)	S → M (3)	
1.992 $\pm$ 0.308	3.079 $\pm$ 0.484	0.984 $\pm$ 0.259	109.1[b] (102.3 ↔ 116.1)

[a] The radioisotopes of sodium were added approximatively 30 minutes after addition of amipramizide, 4×10^{-7} M; 30 more minutes elapsed before flux determinations begun. For each membrane, 3 such flux periods, of 30 minute duration each, were secured. "M" (for mucosa) corresponds to the outside, and "S" (for serosa), to the inside.

[b] Computed from log of individual ratios; not significant different from 100% ($P > 0.1$).

Additional experiments were designed to evaluate the equivalence between sodium flux and short-circuit current when more profound inhibition of the latter was induced by larger amounts of the drug. Observations were made on 5 pairs of bladders, one membrane exposed to 2×10^{-6} M amipramizide while the matched preparation served as a control. Mucosa-to-serosa sodium flux averaged (in μ Eq Na/hour $\pm$ S.E.) 5.100 $\pm$ 0.543 while mean current amounted to 3.903 $\pm$ 0.504 for the control preparations; when the corresponding bladder halves were under the inhibiting influence of amipramizide, the values were 1.323 $\pm$ 0.252 and 0.246 $\pm$ 0.099, respectively. Assuming that the gap between unidirectional flux and net flux is accounted for by sodium movement in the opposite direction (*i.e.* the serosa-to-mucosa flux), it seems that depression of active sodium transport by amipramizide was not associated with changes in the permeability of toad bladder to sodium.

A similar appraisal of a possible disparity between net sodium transport, as measured by short-circuit current, and unidirectional flux was also attempted on toad skin trated with 10^{-5} M amipramizide for a long period of time. There initially was an almost complete suppression of electrical activity, followed by gradual return so that small potentials

Table 2. *Comparison between short-circuit current and sodium flux inward on toad skin treated with amipramizide (Means ± S.E.)*

Incubation conditions	Number of experimental periods	Short-circuit current (μEq Na/hour) (1)	Isotopic flux outside-to-inside (μEq Na/hour) (2)	Difference (2) − (1)
Ringer's	4	0.674 ± 0.081	0.776 ± 0.051	0.102 ± 0.031 ($P < 0.05$)
Chloride-free Ringer's	12	0.516 ± 0.035	0.607 ± 0.060	0.091 ± 0.061 ($P < 0.1$)

and currents could again be read during the fifth and sixth hours of treatment. When outside-to-inside sodium flux was measured isotopically across short-circuited preparations at that stage, there was fairly close agreement between both sets of variables, whether or not chloride was the main anion in the incubation solutions (Table 2).

3. Evidence for Action of Amipramizide at the Apical Border of Toad Bladder and Colon

From the fact that amipramizide depresses active sodium transport quickly, reversibly and only when applied to the outside-facing surface of toad epithelia, the hypothesis was formulated that the "pump", tentatively located at the basal border of the epithelial cells specialized in transcellular sodium transport, would not be directly influenced by amipramizide. Therefore, the following experiments were performed:

a) In 8 instances, the "active sodium transport pool" was measured when toad bladder tissue was exposed to 4×10^{-7} M amipramizide; the size of the pool averaged 60 ± 14 m μ Eq Na while mean sodium transport activity (derived from short-circuit current) amounted to 1.076 ± 0.101 μ Eq Na/hour. From a comparison with values obtained for a series of 50 untreated toad bladders—129 ± 17 m μ Eq Na and 2.040 ± 0.220 μ Eq Na/hour, respectively—it appears that both variables dropped to the same extent under the influence of amipramizide. This is interpreted to indicate that the affinity of the "pump" for sodium was not directly influenced by amipramizide in these circumstances [15].

b) It was tentatively concluded, therefore, that less sodium reaches the "pump" because amipramizide interferes with the transfer of sodium across the apical cell border of the membranes affected. This conclusion was evaluated in a different way by subjecting amipramizide-inhibited epithelia to amphotericin B, a polyene antibiotic that reportedly disrupts the apical diffusion barrier controlling sodium movement from the outside to the "pump" proper [33]. As a consequence of this action of amphotericin B, sodium crosses the apical cell border of toad bladder without the restrictions seen in normal conditions [22]; this was found in this laboratory to apply to the case of toad colon.

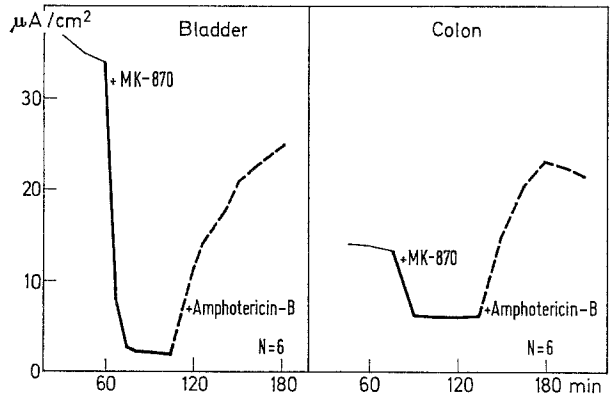

Fig. 6. "Recovery" of short-circuit current upon exposure of amipramizide-treated toad bladder and colon to amphotericin-B. Toad bladder activity was almost abolished by addition to the mucosa-bathing solution of MK-870, 10^{-6} M (——). Activity reappeared after introduction in the solution of amphotericin-B, 2.5 μg/ml (– – –). Short-circuit current across toad colon was less drastically depressed by MK-870, 2×10^{-5} M (——); but it quickly rose to values exceeding the control ones after addition of amphotericin-B, 12.5 μg/ml (– – –)

When amphotericin B was added to the solution to which the mucosal surface of toad bladder or colon was exposed, the antibiotic wiped out, as it were, the inhibiting influence exerted by amipramizide, as illustrated in Fig. 6. In 8 additional paired experiments, bladder halves were treated with 2×10^{-4} M amipramizide, so as to "silence" the membranes; yet electrical activity reappeared if amphotericin B, 5 μg/ml, was added to the amipramizide-containing solution to which matched preparations were exposed: short-circuit current and transmembrane potential averaged 23.0 μA/cm² ± 2.5 and 10.5 mV ± 1.9, respectively. Such a short-circuit current value is analogous to that recorded for a large series of untreated preparations [11].

Conversely, amipramizide was added to membranes already influenced by amphotericin B; hardly any change in short-circuit current resulted as seen on Fig. 7. This again suggests that the presence of the saturable barrier opposing unrestricted diffusion of sodium at the apical cell border, is required for amipramizide to act as inhibitor of active transepithelial sodium transport.

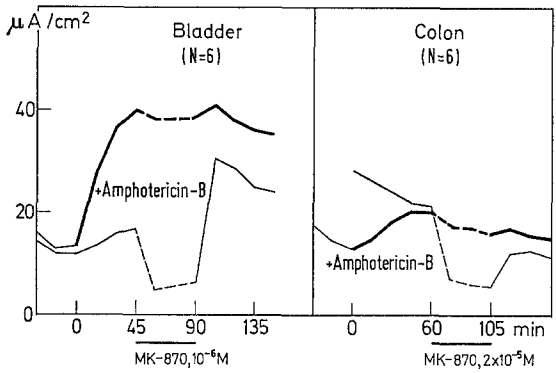

Fig. 7. Ineffectiveness of amipramizide (MK-870) on toad epithelia treated with amphotericin-B. After addition of amphotericin-B to the solution bathing the outside-facing surface of toad bladder and colon, the preparations failed to react significantly to MK-870. In the case of bladder only were experiments carried out on paired halves

4. Hydrodynamic Flow across Toad Bladder Exposed to Amipramizide

The permeability of toad bladder to osmotically-induced water movement was unaltered by amipramizide, 5×10^{-6} M, as demonstrated by experiments performed on 8 pairs of bladder halves submitted to an osmotic gradient of 180 mOsm/kg H_2O. During the second hour of incubation the rate of water flow averaged (in $\mu l/cm^2$/hour) 3.9 ± 0.8 across the control membranes, and 3.5 ± 0.5 for the matched preparations exposed to amipramizide; these values are close to those reported previously by this laboratory for analogous conditions [12]. During the hour following addition of *small* amounts of vasopressin, the permeability to water increased for both sets of preparations, values being 26.4 ± 5.4 and 34.7 ± 11.9, respectively. This difference, of $8.3 \mu l/cm^2$/hour ± 9.4, is not significant statistically ($P > 0.2$).

5. Tissue Composition after Amipramizide Treatment

Twelve paired bladders were employed in this experiment. One of the fragments of each bladder pair was incubated in a medium containing amipramizide, 2×10^{-5} M, for two hours, while the other one was

exposed to a similar incubation medium devoid of amipramizide. No difference could be noted in tissue hydration, or in sodium and potassium contents, when the fragments had been treated with the drug (Table 3).

Table 3. *Influence of amipramizide on water, sodium and potassium contents of toad bladder tissue (Means ± S.E.)*

	Control tissue	Matched tissue exposed to the drug
Tissue water (μl/g dry residue)	324.5 ± 26.7	331.2 ± 27.9
Tissue sodium (μEq/g dry residue)	261.0 ± 13.3	300.8 ± 17.9[a]
Tissue potassium (μEq/g dry residue)	198.2 ± 15.2	198.2 ± 14.2

[a] The increase in tissue sodium content is not significant, statistically ($P > 0.05$).

6. Response of Toad Skin to Hormones during Exposure to Amipramizide

Amipramizide could thus reversibly inhibit active sodium transport by toad bladder, colon and skin, which are epithelia specialized in transcellular sodium movement. Additional studies seemed appropriate to determine whether the inhibitory effect of the drug might be offset or otherwise altered when the rate of transcellular sodium transport had been increased by hormonal stimulation. Ventral skin was utilized for most studies since with this preparation a substantial increase in the rate of sodium transport can be regularly induced *in vitro* by means of aldosterone [13], insulin [2] or vasopressin [42].

In 12 instances, paired fragments of the ventral skin of *Bufo marinus* were incubated overnight with amipramizide, 4×10^{-6} M, present on the outside; one piece of skin of each pair was treated simultaneously with aldosterone. After addition of the inhibitor, short-circuit current (μA/cm² ± S.E.) had dropped from 32.0 ± 7.5 to 4.4 ± 0.6, and from 36.7 ± 7.6 to 4.3 ± 1.3 for matched membranes; the following morning, short-circuit current across the hormone-treated preparations was appreciably larger than was the case for matched controls, values being 16.2 ± 5.0 and 3.8 ± 0.8, respectively. The preparations were not irreversibly damaged by prolonged exposure to amipramizide since currents were 40.8 ± 10.3 and 20.7 ± 7.4, respectively, during the second half-hour after removal of the drug.

In additional experiments, the toad skin preparation was shown to maintain some degree of responsiveness to insulin, and to vasopressin as well, as illustrated by Fig.8; in the case of insulin, the rise was small, yet it was statistically significant ($P < 0.02$). Of interest is the observation that, upon removal of amipramizide, the insulin effect was more apparent; this was not so in the case of vasopressin treatment.

Thus amipramizide failed to prevent toad skin from reacting to aldosterone, antidiuretic hormone or insulin, at least as long as the drug-induced inhibition of sodium transport was not complete.

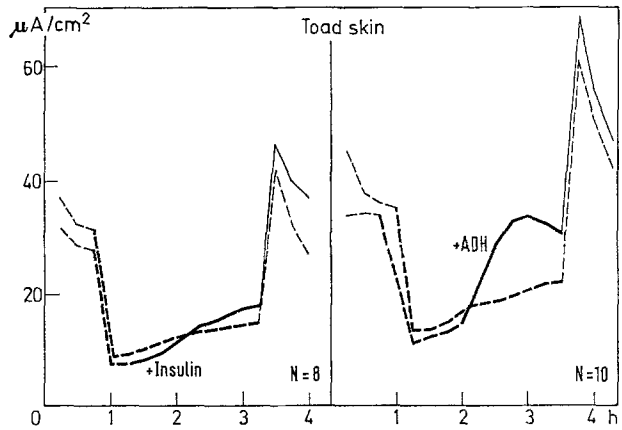

Fig. 8. Stimulation of active sodium transport by toad skin treated with insulin or vasopressin, in the presence of amipramizide (MK-870). After 1 hour of incubation, MK-870, 4×10^{-7} M, was added to the solution bathing the outside of all fragments of ventral skin. Thereafter, one preparation of the pair was exposed to insulin or vasopressin (ADH). Incubation proceeded for 90—120 minutes, where upon the solutions containing MK-870 were replaced with fresh Ringer's. This amplified the insulin effect only

7. Metabolism of Amipramizide in Toad Bladder Epithelium[1]

In 8 instances, the fraction of tissue water accessible to amipramizide was compared to the inulin tissue space which is supposed to yield information on the size of extracellular compartments.

While the inulin space on the outside, measured as described above, averaged $4.2\% \pm 1.2$ of toad bladder tissue water, the value obtained for the same membranes with amipramizide was much larger: $13.9\% \pm 3.2$. The drug might thus accumulate at the surface of the cells or in a compartment located beyond their apical border. Easy diffusion across the preparations was ruled out by the fact that, at the end of incubation, only $0.9‰ \pm 0.09$ of the radioactivity added to the outside compartment could be recovered from the fluid on the inside.

On the other hand the possibility of a tight binding of the drug to toad bladder, already unlikely on account of observations such as illus-

[1] We are grateful to Dr. MANUEL PIZARRO for converting the data obtained by liquid scintillation spectrometry to D.P.M. that were used for calculations.

trated in Fig. 1, was also examined. The mucosal surface of 5 bladders was exposed for more than 15 hours to ^{14}C-amipramizide; the latter was removed and incubation progressed for 1 additional hour, whereupon solutions were sampled for radioactivity, as were eluates of tissue processed as described. Strikingly, the mean amipramizide space was 13.0% ± 3.4 of toad bladder tissue water, despite the fact that the concentration of the drug on the outside was reduced to but one eighth of what it had been during the preceding 15-hour period. This indicates that equilibrium between the tissue and outside-bathing solution is quickly established.

From behaviour on paper chromatography, it seems that most radioactivity recovered from the incubation solutions and from the tissue eluates, still belonged to amipramizide.

Discussion

Amipramizide is a diuretic drug displaying a number of unusual characteristics: (1) Although its natriuretic activity is of limited interest the excretion of hydrogen and potassium ions is decreased as a result of its administration, and it presumably acts upon the distal segments of the renal tubule; (2) It is excreted as such in the urine; (3) As indicated by the data presented above, *in vitro* it exerts an inhibitory effect upon sodium transport by several amphibian epithelia specialized, like the renal tubule, in transcellular sodium transport; (4) This inhibitory effect upon sodium transport was observed only when amipramizide was applied to the outward-facing surface, or apical border, of these amphibian membranes, in which case the effect was practically immediate; remarkably, amipramizide was completely ineffective when applied to the inward-facing (serosal) surface; (5) The degree of inhibition was proportionate to the drug concentration; (6) The inhibiting effect of amipramizide was fully reversible since removal of the drug was followed by an immediate restoration of sodium transport activity to pre-treatment levels.

These observations suggest that amipramizide might act upon the apical border of these membranes, interfering with, or preventing, the entrance of sodium into the cell without binding irreversibly to its receptors. Additional evidence to support this deduction concerning the site of drug action was provided by the fact that the decline in sodium transport induced in toad bladders by amipramizide was associated with a proportionate decrease in the size of the "active sodium transport pool". Furthermore, the inhibition resulting from amipramizide in toad bladder and colon was overcome by simultaneous treatment with amphotericin B, which seemingly eliminates the apical diffusion barrier of these membranes. It would be extremely difficult to ascribe these

findings to a inhibitory effect of amipramizide upon the sodium "pump" that is probably located at the basal border of the cells forming the sodium-transporting layer of these epithelia [9,21,31].

The amphibian epithelia membranes employed in this study resemble each other in that they all have a capacity to achieve a net movement of sodium inward even when the outside concentration of sodium is quite reduced. This is the result of a low rate of passive sodium movement (permeability) in the other direction, combined with a high affinity of the "pump" for sodium. In addition there are arguments suggesting that the apical border of the sodium-transporting cells of these epithelia could be equipped with specific sites with which sodium ions has to combine in order to gain access to the cell interior, thence to the "pump" [8,22].

Of interest—and in keeping with the fact that amipramizide leaves the preparations unharmed—is the observation that the drug did not block hormone-induced increases in sodium transport. This was established for the ventral skin, a preparation capable of such response to the 3 hormones used here: aldosterone, antidiuretic hormone and insulin. The latter would act close to the sodium "pump" [27,36] while the former 2 would instead modify the saturable barrier opposing free diffusion at the apical cell border [16,18,22,49].

It is noteworthy that the three preparations selected for the studies reported here not only have a saturable (for sodium) cell border located apically: they also respond to aldosterone by a stimulation of active transepithelial sodium transport [9,10,13]. Responsiveness to aldosterone and to amipramizide might thus depend on the existence of one and the same structure, namely a saturable diffusion barrier located at the apical border and characterized by the presence of sodium "permeases": under the influence of aldosterone, permease activity would increase, possibly as a result of multiplications of these special molecules. In the presence of amipramizide on the other hand, the capacity of these sodium-specific carriers to move sodium inward, across the apical cell border, would be impaired.

Amipramizide was shown to change the permeability of the toad bladder preparation to water, neither in baseline conditions nor after addition of antidiuretic hormone. Yet, concentration of the latter was purposely kept low so as to be given a chance to evaluate a subtle influence of amipramizide that could have been masked in the presence of supramaximal amounts of hormone [4].

Eigler, Kelter and Renner have concluded in a preliminary report that amipramizide prevented frog skin from reacting to aldosterone and pitressin [20]. The discrepancy between their data and those

discussed here is only apparent since they used sufficient amounts of amipramizide to reduce sodium transporting activity drastically; furthermore, evaluation of hormonal responsiveness is probably more adequate when experiments are carried out on paired preparations—as has been the case here.

Amipramizide is recovered in the urine without undergoing any metabolism [3] .Toad bladder likewise failed to degrade it to any significant extent. On the basis of what is observed in amphibian epithelia, it might be that *in vitro* the drug acts from the tubular lumen, at the apical border of the cells forming the distal tubule, preventing sodium from combining with the hypothetical "permeases". As a consequence the transtubular electrical potential difference would decrease, which would in turn result in a smaller flux of potassium and hydrogen ions from the cell interior into the lumen—hence, to a drop in the rate of excretion of these ions.

It is pertinent to note that the effects of amipramizide on urine composition become apparent 2—4 hours after a single oral dose in normal individuals [26,44], and reaches its maximum during the following 4-hour period. It is precisely the period of time during which the drug can be recovered in largest amounts from the urine [3].

If it were confirmed that amipramizide in the organism acts by virtue of its presence in tubular fluid, exerting its influence at the luminal border of the tubular cells, this would represent a mode of action fundamentally different from that of more potent natriuretic substances which are assumed to act by interfering more or less immediately with the operation of the sodium "pump" itself [19,29,37]. It could be pointed out here that amipramizide is devoid of influence on cell membrane ATPases [3].

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