

# Aldosterone increases the apical Na<sup>+</sup> permeability of toad bladder by two different mechanisms

(epithelial transport/mineralocorticoids/Na<sup>+</sup> channels)

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**ABSTRACT** The aldosterone-induced augmentation of Na<sup>+</sup> transport in toad bladder was analyzed by comparing the hormonal actions on the transepithelial short-circuit current and on the amiloride-sensitive <sup>22</sup>Na<sup>+</sup> uptake in isolated membrane vesicles. Incubating bladders with 0.5 μM aldosterone for 3 hr evoked more than a 2-fold increase of the short-circuit current (because of the activation or insertion of apical amiloride-blockable channels) but had no effect on the amiloride-sensitive Na<sup>+</sup> transport in apical vesicles derived from the treated tissue. A longer incubation (e.g., 6 hr) produced an additional augmentation of the short-circuit current, which was accompanied by about a 3-fold increase of the channel activity in isolated membranes. The stimulatory effect of aldosterone sustained in vesicles was inhibited by the antagonist spironolactone (present at 1000-fold excess) and the protein synthesis inhibitor cycloheximide (1 μM). In addition, triiodothyronine and butyrate, previously reported to partly inhibit the aldosterone-induced increase in short-circuit current, blocked the hormonal effect in vesicles. It is suggested that aldosterone elevates the apical Na<sup>+</sup> permeability of target epithelia by two different mechanisms: a relatively fast effect (≤3 hr), which is insensitive to triiodothyronine or butyrate and is not sustained by the isolated membrane, and a slower or later (>3 hr) response blocked by these reagents, which is preserved by the isolated membrane. The data also indicate that these processes are mediated by different nuclear receptors.

The adrenal steroid aldosterone is a potent regulator of Na<sup>+</sup> reabsorption in tight epithelia such as the distal kidney segments, the urinary bladder, the descending colon, and exocrine glands (1-3). Much of the current knowledge on its mode of action came from studies with the toad urinary bladder, a model tight epithelium in which Na<sup>+</sup> fluxes can conveniently be monitored by recording the transepithelial short-circuit current (*I*<sub>sc</sub>) (4). Such studies have established that aldosterone binding to an intracellular receptor alters gene expression and evokes a 2- to 4-fold increase in the transepithelial Na<sup>+</sup> transport, which develops over a period of several hours (for a review, see refs. 3 and 5). The observed augmentation in *I*<sub>sc</sub> results from at least two different events: (i) an increase in the apical density of "open" Na<sup>+</sup> channels and thereby enhanced rate of luminal Na<sup>+</sup> entry (6) and (ii) an elevated rate of Na<sup>+</sup>/K<sup>+</sup>-ATPase synthesis, which presumably increases the capacity of the basolateral membrane to extrude Na<sup>+</sup> into the interstitial space (7). At short times (<3 hr) the natriuretic action of aldosterone is fully accounted for by the increase in apical Na<sup>+</sup> permeability. Enhanced induction of pump units is apparent only after longer incubation periods (6-8). Although the ability of aldosterone to activate or insert apical channels is well documented, the

molecular events involved are mostly unknown. Data by several groups indicate that at least part of the hormonal action is to activate preexisting nonconductive apical channels rather than induce *de novo* channel synthesis (9-12).

Another unresolved issue is the role of the two different nuclear corticosteroid receptors found in target epithelia (13). An occupancy-response analysis has shown that both the initial increase of apical Na<sup>+</sup> permeability and the enhanced synthesis of Na<sup>+</sup>/K<sup>+</sup>-ATPase are mediated by the binding of aldosterone to a nuclear high-affinity, low-capacity (type I) receptor (14, 15). Nevertheless, the long-term augmentation of Na<sup>+</sup> transport requires at least partial occupancy of a low-affinity, high-capacity (type II) receptor (15).

In this paper we analyze the apical action of aldosterone by comparing the hormone-induced changes in Na<sup>+</sup> transport measured in the intact epithelium with those measured in membrane vesicles derived from it. It is shown that aldosterone increases the apical Na<sup>+</sup> permeability by two independent apical processes, only one of which is sustained by the isolated membrane. These processes differ in their sensitivity to triiodothyronine (T<sub>3</sub>) and butyrate and also may be induced by different nuclear receptors.

## MATERIALS AND METHODS

*Bufo marinus* toads (either sex, Mexican origin, obtained from Lemberger, Oshkosh, WI) were doubly pithed and deblooded by transventricular perfusion with ≈200 ml of NaCl Ringer's solution composed of 110 mM NaCl, 3.5 mM potassium phosphate, 0.5 mM MgCl<sub>2</sub>, and 1 mM CaCl<sub>2</sub>, pH 7.3. The urinary bladders were excised, rinsed well in the above Ringer's solution, and either mounted in Ussing chambers or cut into bladder quarters and immersed in small flasks containing 15 ml of Ringer's solution. Tissues (in Ussing chambers and flasks) were incubated under aeration in the presence or absence of aldosterone and other reagents. Usually the incubating medium was the above NaCl Ringer's solution that contained, in addition, 5 mM glucose, 5 mM pyruvate, and 100 units of penicillin and 100 μg of streptomycin per ml. In some experiments NaCl was substituted by KCl (Na<sup>+</sup>-free medium). Hemibladders mounted in Ussing chambers were used to measure the aldosterone-induced increase in *I*<sub>sc</sub> as described (10). Bladder quarters incubated in flasks were used to evaluate the hormonal action in vesicles. At the end of the incubation, they were rinsed well in an ice-cold homogenizing medium composed of 90 mM KCl, 45 mM sucrose, 10 mM Tris·EGTA, 5 mM MgCl<sub>2</sub>, and 10 mM Tris·HCl, pH 7.8, and the epithelial cells were scraped off the connective tissue with a glass slide. Cells were dispersed by rapidly drawing them in and out of a Pasteur pipette and were washed twice at 0°C by centrifugation (1000

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Abbreviations: *I*<sub>sc</sub>, transepithelial short-circuit current; T<sub>3</sub>, triiodothyronine.

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× *g* for 10 min) and resuspension. Cell aliquots (each aliquot containing cells obtained from a single bladder quarter) were then incubated for 30–45 min at 25°C in the above homogenizing medium. This procedure has been shown to largely increase the channel activity in vesicles (16, 17). Then cells were homogenized, vesicles were isolated, and  $^{22}\text{Na}^+$  uptake was measured in the presence of a negative inside membrane potential ( $\text{K}^+$  gradient + valinomycin) as described (16, 17). The amiloride-sensitive flux (or "channel-activity") is defined as the difference in the initial rate of  $^{22}\text{Na}^+$  uptake in the presence and absence of 1.5  $\mu\text{M}$  amiloride. Its values are expressed in pmol of  $^{22}\text{Na}^+$ ·(mg of protein) $^{-1}$ ·min $^{-1}$  or in relative units (% of the uptake under control conditions).

**Statistics.** All data are expressed as means  $\pm$  SEM, with the number of measurements in parentheses.

**Materials.** Aldosterone, dexamethasone,  $\text{T}_3$ , spironolactone, cycloheximide, valinomycin, and EGTA were from Sigma.  $^{22}\text{NaCl}$  (carrier free; 1.14 mCi/ml; 1 Ci = 37 GBq) was purchased from Amersham Radiochemicals (Buckinghamshire, U.K.), and amiloride was a gift from Merck Sharp & Dohme (Munich).

## RESULTS

Fig. 1 illustrates effects of aldosterone on  $\text{Na}^+$  fluxes in the intact epithelium and in apical membrane vesicles isolated from it. Adding 0.5  $\mu\text{M}$  aldosterone to the serosal compartment of a mounted bladder evoked a typical increase in  $I_{\text{sc}}$  preceded by a latent period of 30–45 min. Previous studies have established that at least the initial (<3 hr) increase in  $I_{\text{sc}}$  is entirely due to an increase in the apical  $\text{Na}^+$  permeability (5–8). The hormone also increased the channel activity in plasma membrane isolated from the treated epithelium, but in this case the latent period was much longer. Incubating bladders with aldosterone for 3 hr increased  $I_{\text{sc}}$  by  $120 \pm 15\%$  but had no significant effect in vesicles (a mean increase of  $18 \pm 12\%$ ).<sup>†</sup> A longer incubation (e.g., 6 hr) produced an additional increase of  $I_{\text{sc}}$ , which was accompanied by >2-fold augmentation of the amiloride-sensitive  $^{22}\text{Na}^+$  uptake in vesicles (Fig. 1). In another nine experiments not included in Fig. 1, incubating bladders with aldosterone for 4 and 16 hr increased the channel activity in vesicles by  $40 \pm 19\%$  and  $165 \pm 20\%$ , respectively. A favorable explanation for the apparent discrepancy between the time course of the hormonal action measured in the intact epithelium and in isolated membrane is that aldosterone increases the apical  $\text{Na}^+$  permeability by two different mechanisms: a relatively fast (or early) process not sustained by the isolated membrane and a slower (or later) event that is preserved by vesicles. According to this interpretation the hormone-induced augmentation of  $I_{\text{sc}}$  sums the two different apical phenomena, while flux measurements in vesicles reflect only the later process.

The experiments summarized in Fig. 2 and Table 1 have established that the increase of  $^{22}\text{Na}^+$  uptake in vesicles is a specific effect of aldosterone, independent of the initial rise in  $I_{\text{sc}}$ . First, it was found that the hormonal pretreatment increases the amiloride-blockable  $^{22}\text{Na}^+$  uptake without affecting the amiloride-insensitive flux (Fig. 2) or the valin-

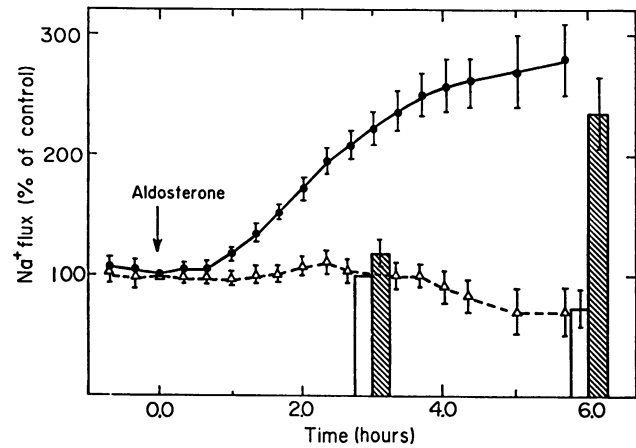


FIG. 1. Aldosterone-induced augmentation of  $\text{Na}^+$  transport in toad bladder and in apical membrane vesicles. Matched hemibladders were mounted as flat sheets in Ussing chambers and preincubated for 8–12 hr at 25°C in aerated NaCl Ringer's solution to deplete the tissue of endogenous aldosterone and to degrade aldosterone-induced proteins. Another pair of hemibladders was cut into four bladder quarters that were incubated under the same conditions in two flasks (two quarters in each flask). At the end of this period, the medium was substituted by fresh Ringer's solution, and 0.5  $\mu\text{M}$  aldosterone was added to one of the chambers and one of the flasks (time zero).  $I_{\text{sc}}$  was measured every 20–40 min in the mounted hemibladders incubated with ( $\bullet$ ) and without ( $\Delta$ ) the hormone. Data are expressed as the percentage of the current at time zero ( $9.3 \pm 2.5 \mu\text{A}/\text{cm}^2$  and  $6.8 \pm 0.8 \mu\text{A}/\text{cm}^2$  for control and aldosterone-treated hemibladders, respectively). The values presented are means  $\pm$  SEM of six experiments. The hormonal action on the isolated membrane was measured as follows: at  $t = 3$  hr, a pair of bladder quarters (aldosterone-treated and control) were removed, and plasma membrane vesicles were prepared and assayed for  $^{22}\text{Na}^+$  uptake as described (16, 17). The same procedure was repeated at  $t = 6$  hr with the other pair. The amiloride-sensitive  $^{22}\text{Na}^+$  uptake in vesicles obtained from aldosterone-treated (hatched bars) and control (open bars) tissue was calculated. Data are expressed as the percentage of the amiloride-sensitive uptake into vesicles derived from the bladder quarter incubated for 3 hr with no additions [ $3.7 \pm 1.5$  pmol of  $^{22}\text{Na}^+$ ·(mg of protein) $^{-1}$ ·min $^{-1}$ ], and means  $\pm$  SEM of eight experiments are depicted.

omycin-mediated  $^{86}\text{Rb}^+$  uptake into the same vesicles (data not shown). Thus, changes in  $^{22}\text{Na}^+$  flux cannot be accounted for by a nonspecific action of the hormonal pretreatment on the yield, size distribution, or membrane potential of apical vesicles. Second, a similar increase of the channel activity was noted in vesicles prepared from bladders incubated with aldosterone in NaCl and Na $^+$ -free (KCl) Ringer's solutions (Fig. 2)—i.e., the slow effect postulated is a primary action of aldosterone and is not secondary to changes in cell  $\text{Na}^+$  during the initial increase in  $I_{\text{sc}}$ . Finally, it was shown that the aldosterone-induced increase of  $\text{Na}^+$  uptake in vesicles is inhibited by the specific antagonist spironolactone and the protein synthesis inhibitor cycloheximide (Table 1). However, cycloheximide lowered the baseline flux by nearly the same factor as the aldosterone-induced flux. Therefore, its antagonizing action may be a nonspecific one.

Previous studies have established that the thyroid hormone  $\text{T}_3$  and butyrate can both antagonize the long-term action of aldosterone without inhibiting the initial increase in  $I_{\text{sc}}$  (7, 18, 19). Butyrate has been shown to block the hormone-induced induction of  $\text{Na}^+/\text{K}^+$ -ATPase (presumably by inhibiting histone deacetylation), but no specific mechanism for the only partial and delayed inhibitory effect of  $\text{T}_3$  was found (7, 19, 20). The current observations raise the possibility that the partial action of  $\text{T}_3$  represents an inhibition of only one of the two independent events that contribute to the increase in  $I_{\text{sc}}$ .

<sup>†</sup>The preparation of  $\text{Na}^+$ -conducting plasma membrane vesicles includes a step in which transport is augmented by incubating scraped cells in a  $\text{Ca}^{2+}$ -free solution (see *Materials and Methods* and refs. 16 and 17). This step is not responsible for the lack of difference between control and aldosterone-treated preparations. Preparing vesicles from cells that were not incubated in a  $\text{Ca}^{2+}$ -free solution gave the same results (but much larger experimental error because of much lower amiloride-blockable fluxes).

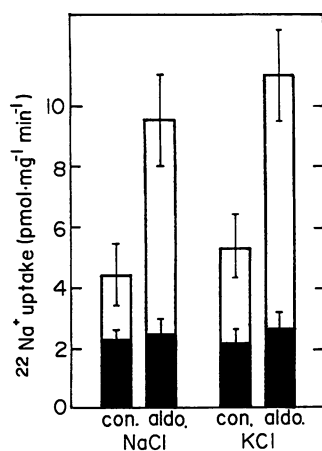


FIG. 2. Effect of the Ringer's solution composition on  $^{22}\text{Na}^+$  uptake in the presence and absence of aldosterone. Bladder quarters were incubated for 16 hr either in NaCl or in  $\text{Na}^+$ -free (KCl) Ringer's solution. The incubation was carried out with (aldo.) and without (con.)  $0.5 \mu\text{M}$  aldosterone. Plasma membrane vesicles were prepared and assayed for  $^{22}\text{Na}^+$  uptake in the presence and absence of amiloride. The amiloride-blockable (empty bars) and amiloride-insensitive (filled bars)  $^{22}\text{Na}^+$  fluxes in vesicles are shown (means  $\pm$  SEM of seven experiments).

Indeed, it was found that the presence of  $10 \text{ nM}$   $\text{T}_3$  during the incubation of bladders with the hormone entirely prevents the aldosterone-induced increase of  $^{22}\text{Na}^+$  uptake in vesicles (Table 1). However, at this concentration  $\text{T}_3$  also lowered the channel activity in membranes that were not exposed to aldosterone. At a lower dose ( $5 \text{ nM}$ ),  $\text{T}_3$  inhibited  $\approx 50\%$  of the response to aldosterone without decreasing the base-line channel activity. Taken together with the previous observation that  $\text{T}_3$  has no effect on the initial (3-hr) rise in  $I_{\text{sc}}$ , it is feasible that this hormone acts specifically on the late natriuretic effect sustained by vesicles. Interestingly enough, butyrate ( $5 \text{ mM}$ ) also inhibited the aldosterone-induced augmentation of  $\text{Na}^+$  transport in vesicles. Thus, this reagent affects not only the induction of  $\text{Na}^+$  pumps but also the late increase in apical conductance.

Next we measured the steroid concentration-dependence of the response measured in vesicles. Aldosterone augmented the amiloride-sensitive tracer flux with an  $\text{IC}_{50}$  of about  $30 \text{ nM}$  (Fig. 3). Dexamethasone, another corticosteroid with high affinity to the aldosterone receptors (21), was equally effective in activating  $^{22}\text{Na}^+$  uptake in vesicles. Comparison of these data to previously reported aldosterone dose-response relationships (14, 15) appears to suggest that the long-term effect measured in vesicles is induced by higher

hormone doses than the concentrations needed to evoke the initial change in  $I_{\text{sc}}$  or tissue resistance. This may indicate that the early and late apical responses are mediated by the occupancy of the high- and low-affinity aldosterone receptors, respectively (13–15, 21). This possibility was assessed by correlating the measured increase in channel activity with the calculated relative occupancy of each receptor. The data summarized in Table 2 clearly show that the increased response to aldosterone correlates with occupancy of type II receptors, while type I receptors are fully occupied at all of the aldosterone concentrations measured. Thus, it appears that the long-term action of aldosterone sustained by vesicles is induced by a different receptor than the one responsible for the early augmentation of  $I_{\text{sc}}$ .

## DISCUSSION

The current paper describes measurements of the  $\text{Na}^+$  channel-mediated tracer fluxes in membrane vesicles derived from toad bladder preincubated with aldosterone and other reagents. Comparing the time course of the hormonal action on the intact tissue and on plasma membrane isolated from it revealed that the well-documented increase in  $I_{\text{sc}}$  sums two different apical processes not distinguished before. The initial ( $\leq 3 \text{ hr}$ ) rise of  $I_{\text{sc}}$ , although reflecting an increase in the density of apical  $\text{Na}^+$  conducting sites, is not preserved by the isolated apical membrane. Therefore, this process must represent a "labile" activation of preexisting channels that reverses as soon as cells are homogenized and their cytoplasmic content is largely diluted. This conclusion is supported by previous data indicating that the aldosterone-induced channels are accessible to impermeable reagents interacting with the apical surface prior to the application of the hormone (9–11). A possible mechanism for such a labile activation of transport could be release of channels from direct inhibition by cell  $\text{Ca}^{2+}$  (22). Incubating the tissue with aldosterone for longer periods (e.g., 6–16 hr) produced an additional increase in  $I_{\text{sc}}$  that was sustained by vesicles. This later, stable augmentation of transport must be mediated by a different mechanism—e.g., *de novo* channel synthesis or the methylation reaction postulated by Sariban-Sohrabay *et al.* (12). In principle it is also possible that the late process does not represent activation or insertion of additional channels but does represent down-regulation of the mechanism that reverses the increase in conductance after cell homogenization.

The notion that the observed increase in  $I_{\text{sc}}$  sums two different apical events provides an explanation for the only partial and delayed antiminerocorticoid action of  $\text{T}_3$  and

Table 1. Antagonists to the stimulatory action of aldosterone

Antagonist	Fractional channel activity, ratio of treated/control samples			
	Aldosterone/control	Antagonist/control	(Aldosterone + antagonist)/control	(Aldosterone + antagonist)/aldosterone
Spironolactone	$2.33 \pm 0.32$ (5)	$0.71 \pm 0.08$ (5)	$0.89 \pm 0.25$ (5)	$0.36 \pm 0.08$ (5)
Cycloheximide	$3.50 \pm 0.60$ (6)	$0.56 \pm 0.10$ (6)	$1.09 \pm 0.27$ (6)	$0.30 \pm 0.07$ (6)
Butyrate	$2.53 \pm 0.15$ (6)	$0.90 \pm 0.18$ (6)	$0.77 \pm 0.10$ (6)	$0.32 \pm 0.06$ (6)
$\text{T}_3$				
10 nM	$2.98 \pm 0.39$ (4)	$0.50 \pm 0.02$ (4)	$1.19 \pm 0.07$ (4)	$0.42 \pm 0.03$ (4)
5 nM	$5.54 \pm 1.36$ (5)	$1.18 \pm 0.33$ (5)	$2.79 \pm 0.92$ (5)	$0.54 \pm 0.10$ (5)

Four matched hemibladders were incubated for 16–18 hr in NaCl Ringer's solution with aldosterone, antagonist, aldosterone + antagonist, or diluent (control). In experiments with spironolactone,  $50 \text{ nM}$  aldosterone was applied. In all other cases the hormone concentration was  $0.5 \mu\text{M}$ . The antagonists used and their concentrations are spironolactone ( $50 \mu\text{M}$ ), cycloheximide ( $1 \mu\text{M}$ ), butyrate ( $5 \text{ mM}$ ), and  $\text{T}_3$  ( $5$  or  $10 \text{ nM}$ ).  $\text{T}_3$  and butyrate were added 2–4 hr before aldosterone; spironolactone and cycloheximide were added together with the hormone. At the end of the incubation, plasma membrane vesicles were prepared and assayed for  $^{22}\text{Na}^+$  uptake. The amiloride-sensitive fluxes in the various preparations are expressed as ratios between treated and control samples. The absolute amiloride-sensitive fluxes under control conditions were (in  $\text{pmol}$  of  $^{22}\text{Na}^+$   $(\text{mg of protein})^{-1} \cdot \text{min}^{-1}$ ): spironolactone,  $3.2 \pm 1.0$ ; cycloheximide,  $5.4 \pm 1.1$ ; butyrate,  $4.4 \pm 0.5$ ;  $10 \text{ nM}$   $\text{T}_3$ ,  $2.0 \pm 0.2$ ; and  $5 \text{ nM}$   $\text{T}_3$ ,  $2.8 \pm 1.0$ . Numbers in parentheses represent numbers of experiments.

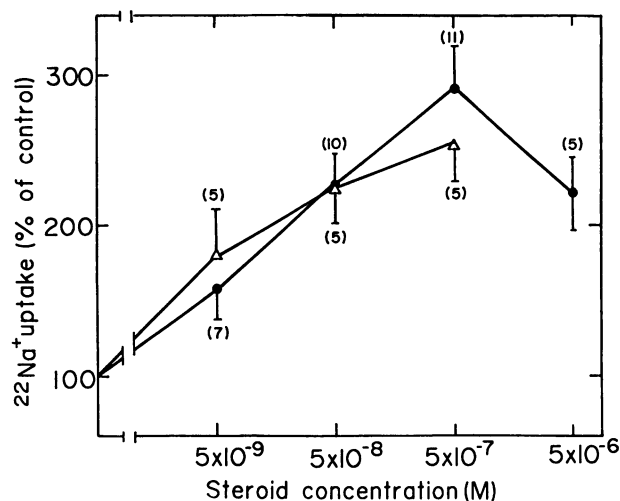


FIG. 3. Steroid dose-response relationships. Four matched bladder quarters were incubated for 16–18 hr in NaCl Ringer's solution in the presence of different concentrations of aldosterone (●) or dexamethasone (Δ). At the end of this period, membrane vesicles were isolated and assayed for  $^{22}\text{Na}^+$  uptake. The amiloride-sensitive fluxes, expressed as the percentage of the uptake in vesicles prepared from matched bladder quarters that were incubated with no added steroids, are plotted against the steroid concentration. Data are presented as means  $\pm$  SEM, with the number of experiments (5–11) in parentheses.

butyrate. Both reagents, previously shown not to affect the initial increase in  $I_{sc}$  (18, 19), blocked the later effect sustained by vesicles. Thus, these reagents specifically inhibit the late apical action of the hormone. The fact that inhibiting histone deacetylase by butyrate inhibits only one of the two aldosterone-induced apical processes indicates that they involve different transcriptional events. It is also interesting to note that both  $T_3$  and butyrate repress the synthesis of a specific aldosterone-induced protein (23). However, the molecular weight of this protein ( $\approx 65$  kDa) differs from those of all amiloride-binding proteins recently identified in tight epithelia (24–27).

The current data also suggest different roles for the two nuclear aldosterone receptors and may account for a number of discrepancies in the literature. It has been reported (15) that both the initial activation of  $\text{Na}^+$  channels (measured as a decrease of the tissue electrical resistance) and the enhanced induction of  $\text{Na}^+/\text{K}^+$ -ATPase correlate with the occupancy of a type I receptor whose  $K_d$  value to aldosterone is  $3 \times 10^{-10}$  M. Nevertheless, the long-term increase in  $I_{sc}$  requires also partial occupancy of a type II receptor whose  $K_d$  value is  $5 \times 10^{-8}$  M. Two nuclear aldosterone receptors with similar  $K_d$  values have been identified in cultured toad

Table 2. Relationships between the increase in channel activity and the occupancy of aldosterone nuclear receptors

Aldosterone, nM	Response, % of maximum	Calculated occupancy of receptors, %	
		Type I	Type II
5	22.7 $\pm$ 7.5 (7)	94.3	9.1
50	57.3 $\pm$ 6.3 (10)	99.4	50
500	100	99.9	90.9

The fractional aldosterone-induced increase in channel activity in vesicles was calculated from the data of Fig. 3 (stimulation by  $0.5 \mu\text{M}$  aldosterone is defined as 100% response) and compared to the calculated occupancies of aldosterone nuclear receptors [ $K_d$  values of  $3 \times 10^{-10}$  M and  $5 \times 10^{-8}$  M were assumed for type I and type II receptors, respectively (15)]. Numbers in parentheses represent numbers of experiments.

kidney (A6) and toad bladder (TB6-C) cells as well (28, 29). In these cell lines, however, the relative increase of  $I_{sc}$  closely resembled occupancy of the type II receptor (28, 29). These discrepancies may be accounted for by assuming that the early and late apical processes postulated in this study are mediated by type I and type II receptors, respectively. This possibility is supported by two findings. First, the data of Fig. 3 correlate best with a  $K_d = 5 \times 10^{-8}$  M (see Table 2). Second, dexamethasone, whose affinity to the type I receptor is lower than that of aldosterone (21), was equally effective in augmenting  $^{22}\text{Na}^+$  uptake. According to the above view, the observed dose-response relationships will depend on the relative contributions of the two processes to  $I_{sc}$  and therefore will vary with time. Thus, after a relatively short incubation with the hormone [e.g., 3–5 hr (14, 15)], the data will fit best the occupancy of a type I receptor, whereas after a prolonged incubation [e.g., 18–24 hr (15, 27, 28)], the response will better correlate with the occupancy of the lower-affinity receptor. It is also possible that the two apical processes have different relative contributions to the hormone-induced increase of  $I_{sc}$  in toad bladder and in cultured epithelia.

Finally, one may speculate on the physiological role of the two different apical mechanisms postulated in the present study. A plausible hypothesis we would like to put forward is that these satisfy a need for acute and chronic natriuretic effects—i.e., a relatively fast and easily reversible response and a slower but longer lasting stimulation of  $\text{Na}^+$  transport.

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