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Effects of 3′Deoxyadenosine and Actinomycin D on RNA Synthesis in Toad Bladder: Analysis of Response to Aldosterone

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Summary. Previous studies have shown that aldosterone increases transepithelial active Na^+ transport after a latent period of about 60 min and incorporation of ^{3}H-uridine into polyadenylated RNA (poly(A)(+)RNA) (putatively poly(A)(+)mRNA) as early as 30 min after aldosterone addition. To assess the physiological importance of this pathway, the effects of 3′deoxyadenosine and actinomycin D were compared in studies on the urinary bladder of the toad *Bufo marinus*. 3′deoxyadenosine (30 μg/ml) only partially, though significantly, inhibited the aldosterone-dependent increase in Na^+ transport measured as short-circuit current (scc). The incorporation of ^{3}H-uridine into poly(A)(+)RNA was inhibited by 70 to 80%. In contrast, Actinomycin D (2 μg/ml) totally inhibited the aldosterone-dependent increase in scc, and the incorporation of ^{3}H-uridine into poly(A)(+)RNA by 68 to 75%. 3′deoxyadenosine or actinomycin D alone had no significant effects on baseline scc, while inhibiting poly(A)(+)RNA to the same extent. The differential effects of deoxyadenosine and actinomycin on aldosterone-dependent Na^+ transport may be related to their different sites of action on RNA synthesis: both drugs inhibited, to a similar extent, cytoplasmic poly(A)(+)mRNA; however, 3′deoxyadenosine, in contrast to Actinomycin D, failed to inhibit poly(A)(−)RNA, sedimenting between 4S and 18S (putatively poly(A)(−)mRNA). We conclude that the mineralocorticoid action of aldosterone during the first three hours depends on the synthesis of both poly(A)(+)mRNA and poly(A)(−)mRNA.

Key words: poly(A)(+)mRNA, poly(A)(−)mRNA.

In previous studies, it has been shown that aldosterone increased the incorporation of ^{3}H-uridine into 9–12s cytoplasmic RNA of the toad bladder during the latent period with respect to Na^+ transport (Rossier, Wilce & Edelman, 1974). This effect was independent of the acid soluble pool of ^{3}H-uridine and was mineralocorticoid specific (Rossier, Wilce & Edelman, 1977*a*). Evidence was also obtained that polyadenylated RNA (i.e., poly(A)(+)RNA) was induced during this period (Wilce, Rossier & Edelman, 1976; Rossier *et al.*, 1977*a*). These findings were consistent with earlier studies which showed that actinomycin D (Edelman, Bogoroch &

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Porter, 1963) and 3'deoxyadenosine (cordycepin) (Chu & Edelman, 1972) were both effective inhibitors of aldosterone-dependent Na^+ transport. At high concentration (i.e., 2 μg/ml and above), actinomycin D inhibits the transcription of all major classes of RNA (rRNA, tRNA and mRNA) (Reich, 1963). In contrast, 3'deoxyadenosine selectively blocks rRNA synthesis by inhibiting the RNA polymerase I (Siev, Weinberg & Penman, 1969) but not the transcription of heterogeneous nuclear RNA (HnRNA) which contains probably the precursors for both poly(A)(+)mRNA and poly(A)(−)mRNA (Adesnik *et al.*, 1972; Jelinek *et al.*, 1973). In addition 3'deoxyadenosine inhibits the appearance of poly(A)(+)mRNA into polysomes presumably by inhibiting the nuclear polyadenylation step (an early post-transcriptional event) (Penman, Rosbash & Penman, 1970). The synthesis of poly(A)(−)mRNA such as histone mRNA (Breindl & Gallwitz, 1974) is not inhibited. In the present study, we took advantage of this differential effect to assess further the role of the synthesis of poly(A)(+) and poly(A)(−)mRNA in the response to aldosterone.

Materials and Methods

The incubation medium (frog Ringer's solution) contained (in mM): 90 NaCl, 3 KCl, 25 $NaHCO_3$, 0.5 $MgSO_4$, 0.5 KH_2PO_4, 1 $CaCl_2$ and 6 glucose; pH = 7.5 (gassed with 5% CO_2), osmolality = 230 mosmol. Gentamicin was added to a final concentration of 5 μg/ml, and the medium was filtered through 0.22 μm Millipore filters. All glassware and other solutions were heat sterilized. D-aldosterone was obtained from Calbiochem Corp. and oligo-deoxythymidylate cellulose (T2) (oligo-dT)-cellulose from Collaborative Research, Inc. Actinomycin D (Cosmegen®) was from Merck Sharp and Dohme International and cordycepin (3'deoxyadenosine) from Sigma. All of the conventional reagents were either reagent grade or spectroquality. Instagel® was from Packard Inc. 5-^{3}H-uridine (30 Ci/mM) was purchased from the Radiochemical Center, Amersham.

Colombian male and female toads *(Bufo marinus)* from Charles P. Chase Co., Inc., Miami, Florida, were partially immersed in saline (0.050 M) at 25 °C (±1 °C) for 5 to 6 days before use. After double pithing and perfusion of the circulation with about 200 ml of oxygenated frog Ringer's solution, hemibladders (mucosal side outside) were mounted as sacs on glass cannulas, filled with 5 ml and immersed in 100 ml of the frog Ringer's solution. Both sides were oxygenated with 95% O_2 − 5% CO_2, and the temperature of the bath was maintained at 25 °C (±0.1 °C). The transepithelial electrical potential difference (PD) and the short-circuit current (scc) were measured at 30-min intervals as described previously (Rossier *et al.*, 1974). Three to four hours after mounting, the inhibitor 3'deoxyadenosine or actinomycin D) was added to the serosal medium of one of the hemibladders. Three protocols were used: (i) inhibitor (test) *vs.* diluent (control), (ii) inhibitor + aldosterone (test) *vs.* aldosterone (control), (iii) inhibitor + aldosterone (test) *vs.* inhibitor 3'deoxyadenosine or actinomycin D was added to the serosal medium of one of 30 min, respectively, before addition of aldosterone. These preincubation periods were found to be adequate to obtain steady-state level of inhibition. Based on dose response curves for the inhibitors, 3'deoxyadenosine was used at a final concentration of 30 μg/ml

and actinomycin D at 2 μg/ml. At t_0, aldosterone was added to the mucosal and serosal media (final concentration $= 7 \times 10^{-8}$ M). At the same time ^{3}H-uridine (2 μCi/ml) was added to the serosal medium of both the control and test preparations. The incubations were terminated 180 min later. To establish the dose-response curve (effects of the inhibitor on poly(A)(+)RNA synthesis) homologous pools of tissue (quarter of bladders) were incubated without mounting on glass cannulas in Erlenmeyer flasks containing 15 ml of frog Ringer's solution at 25 °C, oxygenated, and shaken constantly in a Grant incubator. The effects of actinomycin D was studied from 0.5 to 10 μg/ml and that of 3'deoxyadenosine from 7.5 to 90 μg/ml.

Isolation of cytoplasmic RNA

At the end of the incubation period, the media were discarded and the hemibladders were washed twice with ice-cold frog Ringer's solution, and all subsequent steps were carried out at 0–4 °C. Epithelial cells were collected by scraping with a glass slide. The scrapings of 5 or 10 paired hemibladders (or 12 quarters of bladders) were pooled into test and control pools. These pools were homogenized with 15 strokes at 2000 rpm in a motor-driven, Teflon-glass, Potter-Elvehjem homogenizer. Cytoplasmic RNA was isolated by a modification (Rossier *et al.*, 1974) of Penman's procedure (Penman, 1966). Poly(A)(+)RNA of the cytoplasmic extracts was collected by (oligo-dt)-cellulose chromatography (Wilce *et al.*, 1976). In some experiments, the material which did not bind to the column (i.e., poly(A)(−)RNA containing tRNA, rRNA and poly(A)(−)mRNA) was precipitated by 2 volumes of ethanol at −20 °C before being reextracted once on a sodium-dodecyl-sulfate-phenol mixture at 25 °C for 5 min as described in an earlier publication (Rossier *et al.*, 1974; Wilce *et al.*, 1976).

Sucrose gradient analysis of RNA

Equal amounts of test or control cytoplasmic RNA, 1 A_{260} Unit (1 A_{260} Unit = 32 μg RNA), or poly(A)(+)RNA, 0.03–0.04 A_{260} Units were layered on 5 ml, 5–20% linear sucrose gradients and centrifuged at 45,000 rpm in the SW 50.1 Beckman rotor on the Sorwall OTD2 ultracentrifuge at 4 °C for 210 min. Thirty fractions were collected with the Isco model 183 gradient fractionator monitored at 254 nm (UA-5 Absorbance Monitor). Each fraction (~160 μl) was diluted with water up to 800 μl final volume, 2.7 ml Instagel® solution was added to the 4-ml vial, and the mixture was shaken until the gel was formed. ^{3}H content was assayed in a Tri Carb liquid scintillation spectrometer with an efficiency of ~40%. The recovery of radioactivity from the gradients varied from 90 to 95%.

Statistics: The significance of the differences in the mean values was estimated by the paired Student-*t*-test (Snedecor & Cochran, 1967).

Results

Effects of Actinomycin D and 3'Deoxyadenosine on the Synthesis of Poly(A)(+)RNA

To assess the effects of actinomycin D and 3'deoxyadenosine on the incorporation of ^{3}H-uridine into poly(A)(+)RNA, a dose-response curve was studied in homologous pools of 10 to 12 quarters of bladders

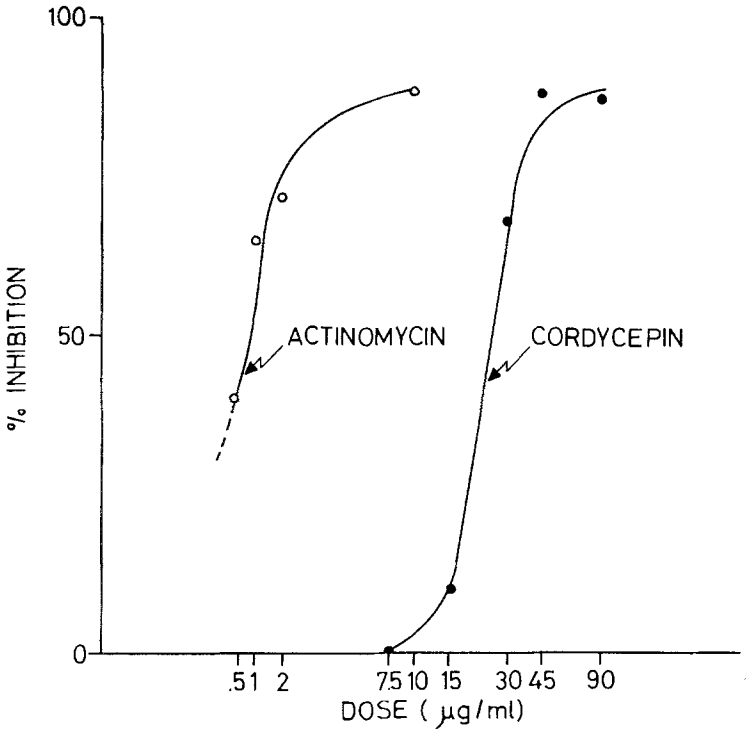

Fig. 1. Actinomycin D and cordycepin (3′deoxyadenosine) dose-response curves for the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Quarters of bladders ($n=12$) were preincubated in Erlenmeyer flasks (in 15 ml of frog Ringer's) in the presence of diluent at various concentrations of inhibitor for 30 min (actinomycin D) or for 60 min (3′deoxyadenosine). All quarters of bladders were then continuously labeled with ^{3}H-uridine for 180 min. Cytoplasmic poly(A)(+)RNA was prepared by (oligo-dt)-cellulose chromatography. The *abscissa* indicates the concentration of inhibitors in µg/ml on a logarithmic scale. The *ordinate* indicates the inhibition of ^{3}H-uridine incorporation into poly(A)(+)RNA as percentage of control values

incubated in Erlenmeyer flasks and labeled with ^{3}H-uridine for 180 min in the presence of various concentrations of inhibitors. Following cytoplasmic extraction of RNA, poly(A)(+)RNA was isolated by (oligo-dT)-cellulose chromatography; specific activity was measured in this fraction.

As shown in Fig. 1, Actinomycin D inhibited 90% of the poly(A)(+)RNA synthesis at a concentration of 10 µg/ml, and 3′deoxyadenosine (cordycepin) inhibited 90% of poly(A)(+)RNA synthesis at a concentration of 90 µg/ml. However, in both instances, these doses of inhibitors were found (data not shown) to cause a significant drop in the baseline scc. For this reason a concentration of actinomycin D (2 µg/ml) and 3′deoxyadenosine (30 µg/ml) were chosen for further studies. These

concentrations yielded an equivalent degree of inhibition (i.e., 70 to 75%) of the poly(A)(+)RNA synthesis and did not significantly affect scc baseline *(see below)*.

Effects of 3'Deoxyadenosine on Na Transport and Poly(A)(+)RNA

3'Deoxyadenosine *vs.* Control

To assess the effects of 3'deoxyadenosine alone on Na^+ transport and poly(A)(+)RNA synthesis, paired hemibladders were incubated continuously with the inhibitor for 240 min. At the end of the period of incubation, the scc was slightly, but not significantly, lower in the hemibladders exposed to 3'deoxyadenosine (Fig. 2, upper panel): the $\Delta\mu A$ (i.e., $scc_{240} - scc_0 \pm SEM$) of the control hemibladders was $+52 \pm 29\,\mu A$ and the test $+30 \pm 35\,\mu A$ ($n=11$; $P>0.3$).

Cytoplasmic poly(A)(+)RNA was labeled continuously for 180 min with ^3H-uridine and analyzed on sucrose gradient (Fig. 2, lower panel). Incorporation of ^3H-uridine was markedly inhibited by 3'deoxyadenosine, especially for those classes of poly(A)(+)RNA sedimenting between 4*s* and 20*s*. In the 12*s* region (Table 1), which represents one of the classes of RNA induced by aldosterone (Rossier *et al.*, 1974; Wilce *et al.*, 1976), the degree of inhibition was, on average, 73%.

3'Deoxyadenosine + Aldosterone *vs.* Aldosterone

If induction of poly(A)(+)RNA synthesis plays a significant role in the effect of aldosterone on Na^+ transport, 3'deoxyadenosine should be an effective inhibitor of the transport response. This hypothesis was tested by preincubating one member of each pair of hemibladders with 3'deoxyadenosine (30 μg/ml) for 60 min followed by subsequent addition of aldosterone (7×10^{-8} M) to both. The results in Fig. 3 (upper panel) indicate that the scc of the group pretreated with 3'deoxyadenosine responded significantly less to aldosterone ($\Delta\,\mu A = 91 \pm 30\,\mu A$) than the group which received aldosterone alone ($\Delta\,\mu A = 215 \pm 61\,\mu A$). This difference was significant at 180 min ($n=11$, $P<0.05$). The profiles of ^3H-uridine incorporation into cytoplasmic poly(A)(+)RNA is shown in Fig. 3 (lower panel). The degree of inhibition in the 12*s* region (Table 1) averaged 80%.

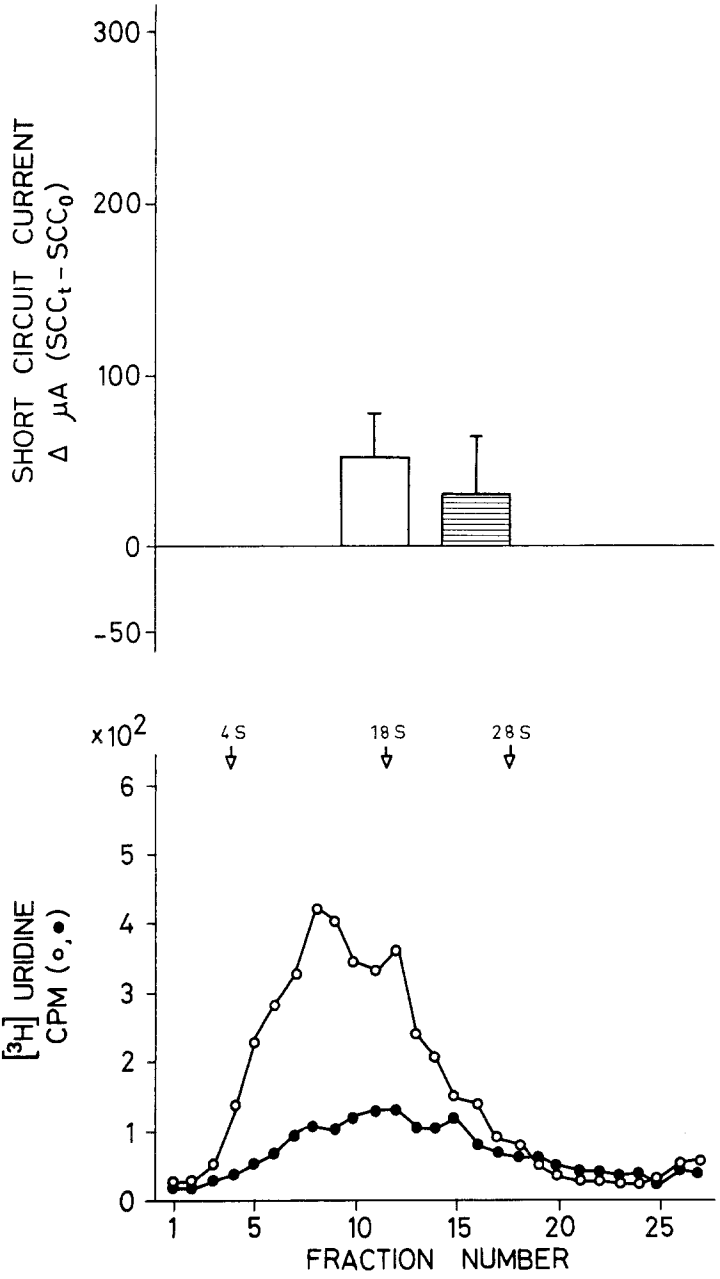

Fig. 2. Effects of 3′deoxyadenosine on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladders ($n=11$) were incubated in 3′deoxyadenosine (test) or the diluent (control) for 60 min. All hemibladders were then labeled continuously with ^{3}H-uridine (2 μCi/ml) for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc per hemibladder. Δ μA (i.e., $scc_{240} - scc_0$) was measured in the individual hemibladder. *Open bar:* control ± SEM. *Hatched bar:* test ± SEM. The difference at 240 min is not significant with a paired-*t*-test analysis ($P > 0.3$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradients. ^{3}H-activity of the control is denoted by -o- and of 3′deoxyadenosine by -●-. Sedimentation was from left to right. *s* values were determined by reference to 4*s*, 18*s* and 28*s* peaks of cytoplasmic RNA centrifuged in parallel

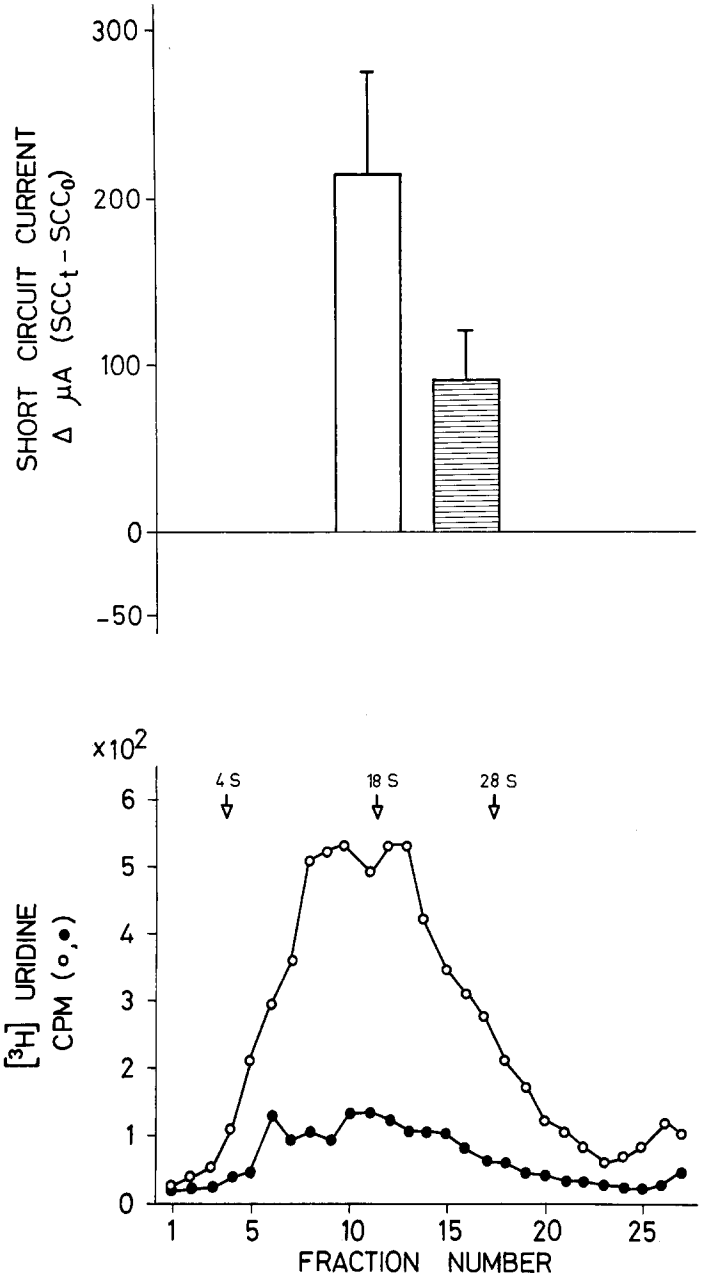

Fig. 3. Effects of 3′deoxyadenosine and aldosterone on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladders ($n=11$) were preincubated in 3′deoxyadenosine (30 μg/ml) (test) or the diluent (control) for 60 min, and then aldosterone (7×10^{-8} M) was added to both (t_0). The hemibladders were then continuously labeled with ^{3}H-uridine for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc per hemibladder. Δ μA (i.e., $scc_{180}-scc_0$) was measured in the individual hemibladders. *Open bar:* control ± SEM. *Hatched bar:* test ± SEM. The difference at t_{180} is significant by paired-*t*-test ($P<0.05$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradients. ^{3}H-activity of the aldosterone-treated group (control) is denoted by -○- and aldosterone + 3′deoxyadenosine-treated group (test) by -●-. *s* values were determined as in Fig. 2

Table 1. Effects of 3′deoxyadenosine and aldosterone on the incorporation of ^{3}H-uridine into cytoplasmic poly(A)(+)RNA analyzed on sucrose gradients

Addition	Cytoplasmic "12*s*" poly(A)(+)RNA (cpm)	Test/Control
a) Diluent	1155	0.27
3′deoxyadenosine	310	
b) Aldosterone	1560	0.20
3′deoxyadenosine + aldosterone	315	
c) 3′deoxyadenosine	375	1.04
3′deoxyadenosine + aldosterone	395	

Pairs of hemibladders were preincubated in (*a*) 3′deoxyadenosine (30 μg/ml) or the diluent for 60 min ($n=11$ pairs of hemibladders), (*b*) challenged with aldosterone (7×10^{-8} M) in the presence of 3′deoxyadenosine or the diluent ($n=11$ pairs of hemibladders), (*c*) challenged with aldosterone (7×10^{-8} M) or the diluent in the presence of 3′deoxyadenosine in both sets of hemibladders ($n=11$ pairs). All hemibladders were continuously labeled with ^{3}H-uridine for 180 min. Cytoplasmic poly(A)(+)RNA was prepared and analyzed on sucrose gradients. ^{3}H-activity in the three fractions sedimenting at 12*s* is shown. *s* value was determined by reference to 4*s*, 18*s* and 28*s* peaks of unfractionated cytoplasmic RNA centrifuged in parallel.

Aldosterone + 3′Deoxyadenosine *vs.* 3′Deoxyadenosine

The results summarized above indicate that the action of aldosterone on Na^+ transport is impaired by 3′deoxyadenosine. Presumably this effect is related to the inhibition of poly(A)(+)mRNA synthesis. However, it is apparent that a part of the response on Na^+ transport was not sensitive to the inhibitor. To further explore this finding, paired hemibladders were preincubated with 3′deoxyadenosine (30 μg/ml) for 60 min, and one member of each pair was then challenged with aldosterone (7×10^{-8} M). As shown in Fig. 4 (upper panel), the scc of the 3′deoxyadenosine + aldosterone-treated hemibladders ($\Delta\,\mu A = scc_{180} - scc_0 = +110 \pm 34\,\mu A$) was significantly higher than the 3′deoxyadenosine-treated hemibladders ($\Delta\,\mu A = scc_{180} - scc_0 = -1 \pm 18\,\mu A$) ($n=11$; $P<0.01$). In contrast, the profile of incorporation of ^{3}H-uridine into poly(A)(+)RNA were almost indistinguishable (Fig. 4, lower panel), and the difference in the 12*s* region was only +4% (Table 1) in the aldosterone group. Thus, in the presence of an almost identical degree of inhibition of poly(A)(+)RNA, a significant response to aldosterone on scc could be observed.

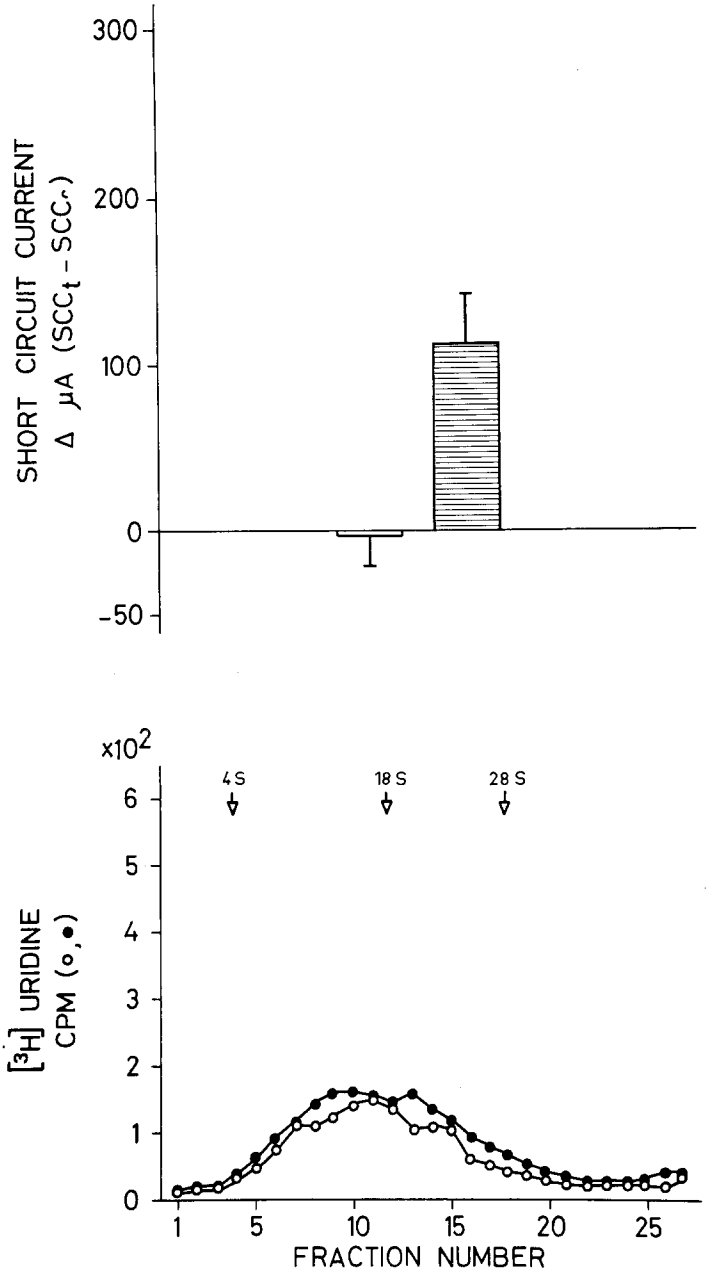

Fig. 4. Effects of aldosterone and 3′deoxyadenosine on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladder ($n=11$) were preincubated in 3′deoxyadenosine (30 μg/ml) for 60 min, and then aldosterone (7×10^{-8} M) (test) was added to one of each pair (t_0) and diluent (control) to the other. The hemibladders were then labeled continuously with ^{3}H-uridine for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc per hemibladder. Δ μA($scc_{180} - scc_0$) was measured in the individual hemibladders. *Open bar:* control ± SEM. *Hatched bar:* test ± SEM. The difference at 180 min is significant ($P<0.01$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradients. ^{3}H-activity of the aldosterone + 3′deoxyadenosine group (test) is denoted by -●- and 3′deoxyadenosine group (control) by -o-. *s* values were determined as in Fig. 2

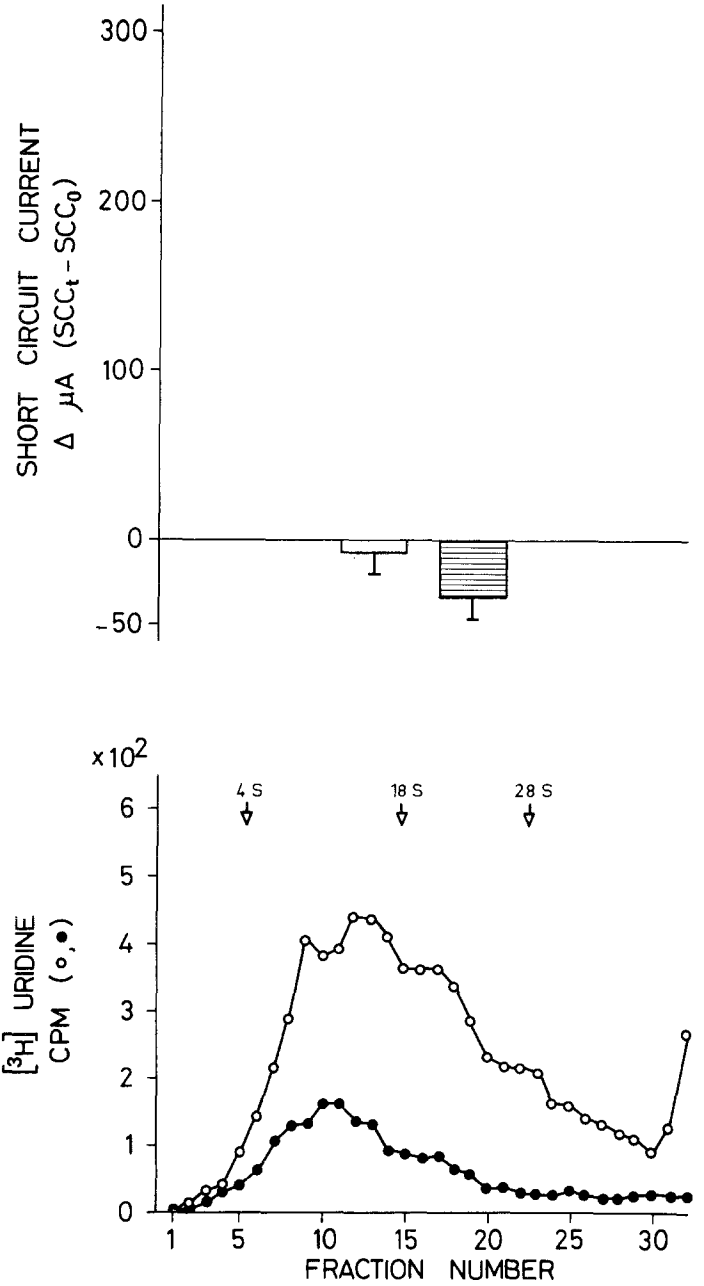

Fig. 5. Effects of actinomycin D on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladders ($n=9$) were incubated in actinomycin D (test) as the diluent (control) for 30 min. All hemibladders were then labeled continuously with ^{3}H-uridine (2 μCi/ml) for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc per hemibladder. Δ μA(i.e., $scc_{240} - scc_0$) was measured in the individual hemibladders. *Open bar:* Control ± SEM. *Hatched bar:* test ± SEM. The difference at 210 min is not significant with a paired-*t*-test analysis ($P > 0.1$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradients. ^{3}H-activity of the control is denoted by -o- and of actinomycin D by -●-. Sedimentation was from left to right. *s* values were determined as in Fig. 2

Effects of Actinomycin D on Na^+ Transport and RNA Metabolism

As cordycepin does not inhibit poly(A)(−)RNA, it was conceivable that a coordinate synthesis of poly A(+) and poly(−)mRNA was necessary in order to obtain the full effect of aldosterone on Na^+ transport.

By the use of actinomycin D which blocks the synthesis of both classes of mRNA, this hypothesis was tested indirectly.

Actinomycin D *vs.* Control

To assess the effects of actinomycin D (2 μg/ml) on Na^+ transport and RNA metabolism, paired hemibladders were incubated continuously with the inhibitor for 210 min. At the end of the incubation the scc was slightly, but not significantly, lower in the hemibladders exposed to actinomycin D (Fig. 5, upper panel). The $\Delta\,\mu A(scc_{240}-scc_0)$ of the control hemibladders was -4 ± 11 μA, and that of the actinomycin-treated hemibladders -35 ± 11 μA ($n=9$; $P>0.1$).

As shown in Fig. 5 (lower panel), cytoplasmic poly(A)(+)RNA labeled continuously for 180 min with ^{3}H-uridine was analyzed on sucrose gradients. The degree of inhibition in the 12 *s* region averaged 68 % and, thus, was similar to that observed with 3′deoxyadenosine (30 μg/ml) (Table 2).

Table 2. Effects of actinomycin D and aldosterone on the incorporation of ^{3}H-uridine into cytoplasmic poly(A)(+)RNA analyzed on sucrose gradients

Addition	Cytoplasmic "12*s*" poly(A)(+)RNA (cpm)	Test/Control
a) Diluent	1235	0.32
Actinomycin D	395	
b) Aldosterone	1510	0.25
Actinomycin D + aldosterone	375	
c) Actinomycin D	380	1.16
Actinomycin D + aldosterone	440	

Pairs of hemibladders were preincubated in either (*a*) actinomycin D (2 μg/ml) or the diluent for 30 min ($n=9$ pairs of hemibladders) or (*b*) challenged with aldosterone (7×10^{-8} M) in the presence of actinomycin D or the diluent ($n=10$ pairs of hemibladders) or (*c*) challenged with aldosterone or the diluent in the presence of actinomycin D ($n=9$ pairs). All hemibladders were continuously labeled with ^{3}H-uridine for 180 min. Cytoplasmic poly(A)(+)RNA was prepared and analyzed on sucrose gradients. ^{3}H-activity in the three fractions sedimenting at 12*s* is shown. *s* value was determined by reference to 4*s*, 18*s* and 28*s* peaks of unfractionated cytoplasmic RNA centrifuged in parallel.

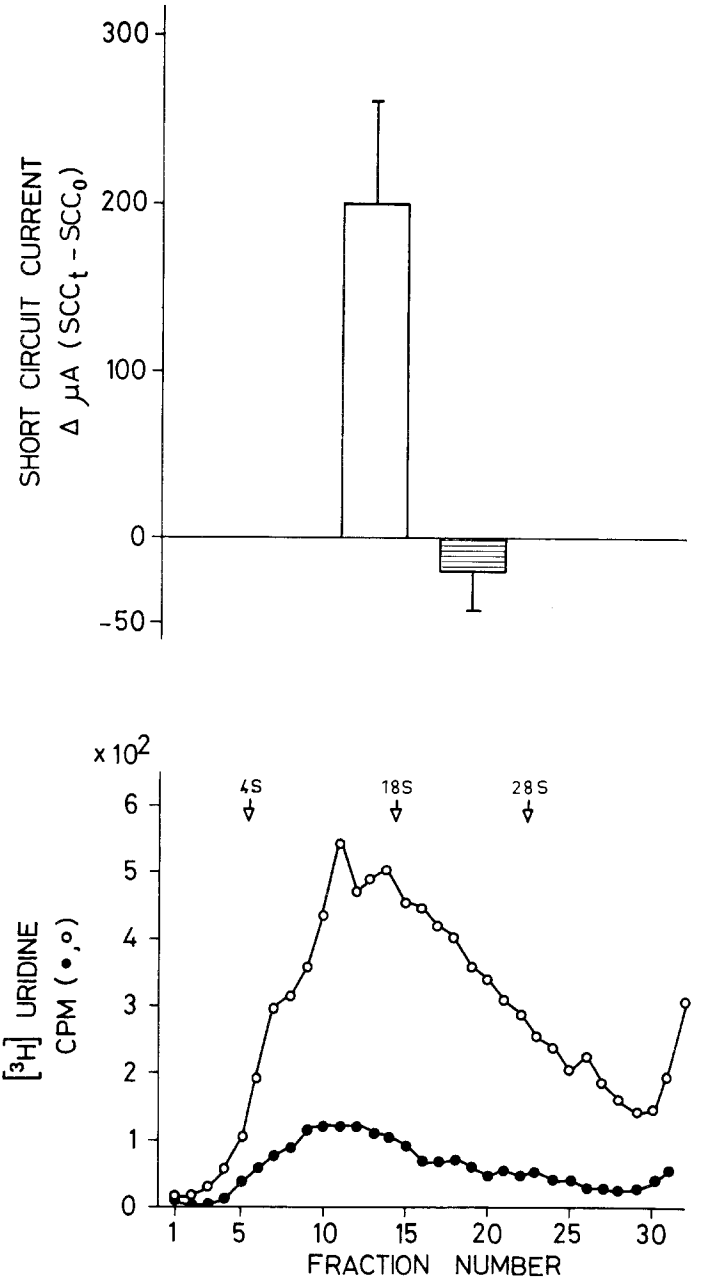

Fig. 6. Effects of actinomycin D and aldosterone on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladders ($n=10$) were preincubated in actinomycin D (2 μg/ml) (test) or the diluent (control) for 60 min, and then aldosterone (7×10^{-8} M) was added to both (t_0). The hemibladders were then labeled continuously with ^{3}H-uridine for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc per hemibladder. Δ μA(i.e., $scc_{180}-scc_0$) was measured in the individual hemibladders. *Open bar:* control ± SEM. *Hatched bar:* + test ± SEM. The difference at 180 min is significant by paired-*t*-test analysis ($P<0.01$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradients. ^{3}H-activity of the aldosterone-treated group (control) is denoted by -○- and aldosterone + actinomycin D group (test) by -●-. *s* values were determined as in Fig. 2

Actinomycin D + Aldosterone *vs.* Aldosterone

If synthesis of both poly(A)(+)RNA and poly(A)(−)RNA plays a significant role in the effect of aldosterone on Na^+ transport, actinomycin D should be a more effective inhibitor of the transport response than 3′deoxyadenosine. This prediction was evaluated by preincubating one member of each pair of hemibladders with actinomycin D (2 μg/ml) for 30 min followed by addition of aldosterone (7×10^{-8} M) to both. The results in Fig. 6 (upper panel) indicate that the scc of the aldosterone-treated group differed significantly at 180 min ($\Delta\,\mu A = scc_{240} - scc_0 = +221 \pm 64\,\mu A$) from the group pretreated with actinomycin D ($\Delta\,\mu A = -21 \pm 25\,\mu A$) ($n = 10$; $P < 0.01$).

The profile of ^{3}H-uridine incorporation into cytoplasmic poly(A)(+)RNA is shown in Fig. 6 (lower panel). The degree of inhibition in the 12 *s* region averaged 75% (Table 2) and, thus, was similar to that observed with 3′deoxyadenosine.

Aldosterone + Actinomycin D *vs.* Actinomycin D

These results imply that the action of aldosterone on Na^+ transport is completely inhibited by actinomycin D but not by 3′deoxyadenosine. To be sure that no further response could be elicited in the presence of a 70 to 80% inhibition of poly(A)(+)RNA synthesis, paired hemibladders were preincubated with actinomycin D (2 μg/ml) for 30 min, and one member of each pair was then challenged with aldosterone (7×10^{-8} M). As shown in Fig. 7 (upper panel), the scc of the actinomycin D + aldosterone-treated hemibladders ($\Delta\,\mu A = scc_{180} - scc_0 = +9 \pm 6\,\mu A$) did not differ significantly from that of the actinomycin D-treated hemibladder ($\Delta\,\mu A = scc_{180} - scc_0 = -14 \pm 18\,\mu A$) ($n = 9$; $P > 0.2$).

In parallel, the profiles of incorporation of ^{3}H-uridine into poly(A)(+)RNA were similar, as shown in Fig. 7 (lower panel). The difference of incorporation averaged 16% in the 12 *s* region of the gradient (Table 2).

Differential Effect of 3′Deoxyadenosine and Actinomycin D on Poly(A)(−)RNA Synthesis

The explanation for a differential effect of the two inhibitors on the physiological response to aldosterone may lie in their differential effects on HnRNA, the precursor of both poly(A)(+) and poly A(−)mRNA. Therefore, the effect of the two inhibitors on poly(A)(−)RNA synthesis

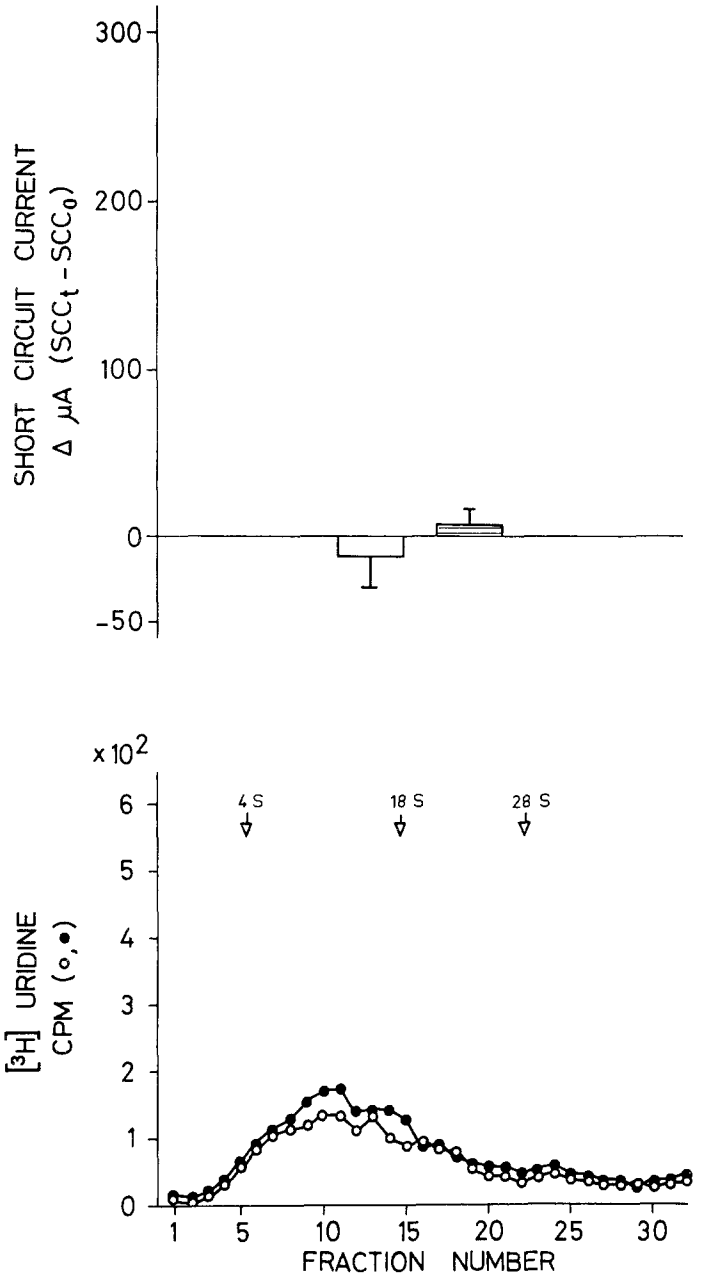

Fig. 7. Effects of aldosterone and actinomycin D on Na^+ transport and on the incorporation of ^{3}H-uridine into poly(A)(+)RNA. Paired hemibladders ($n=9$) were preincubated in actinomycin D (2 μg/ml) for 30 min, and then aldosterone (7×10^{-8} M) (test) was added to one of each pair (t_0) and diluent to the other (control). The hemibladders were then labeled continuously with ^{3}H-uridine for 180 min. *Upper panel:* Net flux of Na^+ transport was measured as the scc in the individual hemibladders. *Open bar:* control ±SEM. *Hatched bar:* test ±SEM. The difference at 180 min is not significant ($P>0.3$). *Lower panel:* Poly(A)(+)RNA analyzed on sucrose density gradient. ^{3}H-activity of the aldosterone + actinomycin D group (test) is denoted by -●- and actinomycin D group (control) by -o-. *s* values were determined as in Fig. 2

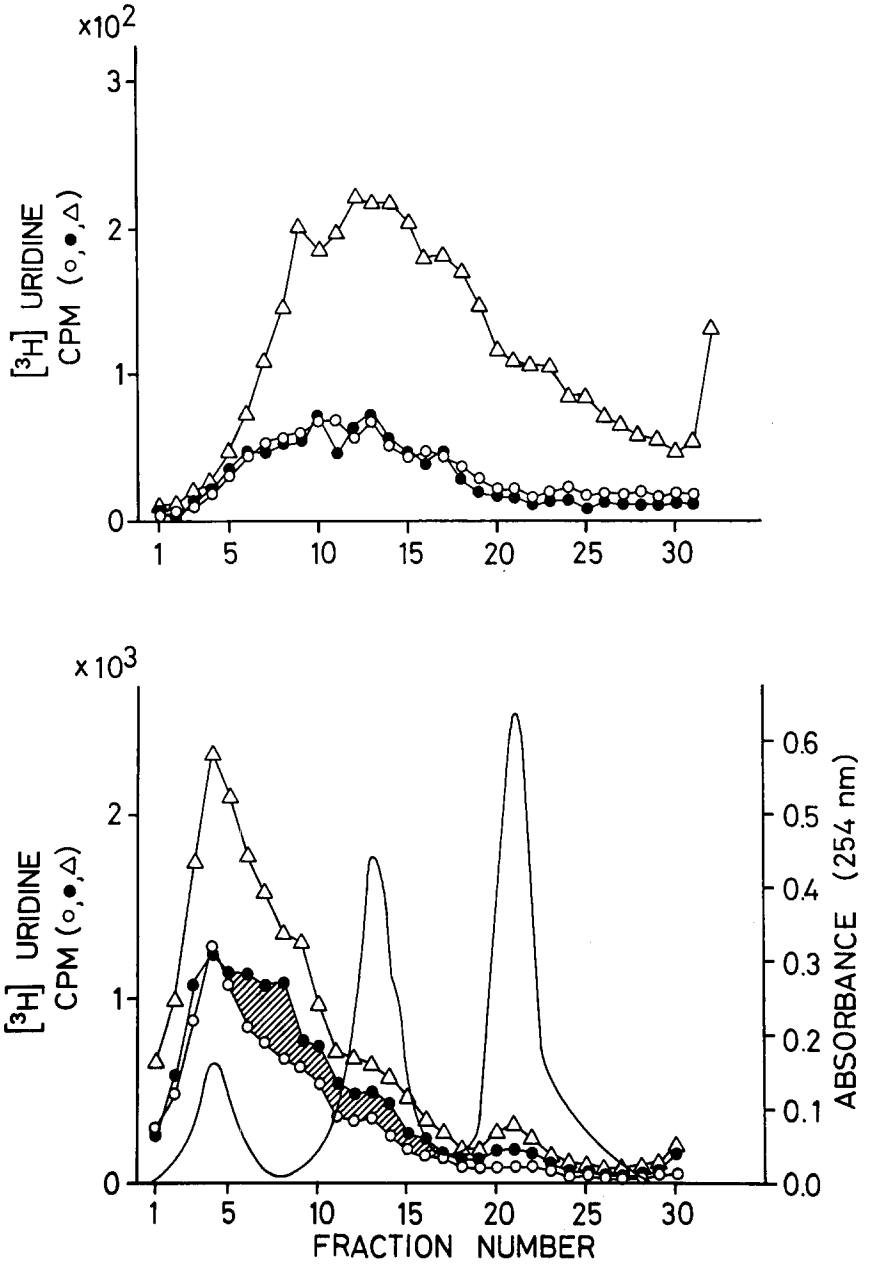

Fig. 8. Differential effect of actinomycin D and 3′deoxyadenosine on the incorporation of ^{3}H-uridine into poly(A)(+)RNA (upper panel) and poly(A)(−)RNA (lower panel). Conditions were as described in legend of Fig. 1. Poly(A)(+)RNA was obtained by (oligo-dT)-cellulose chromatography (low salt eluate). Poly(A)(−)RNA, i.e., unbound material (high salt eluate), was obtained as described in *Materials and Methods. Upper panel:* Poly(A)(+)RNA analyzed on sucrose gradients. *Left abscissa:* -△- denotes ^{3}H-activity of control; -●-, 3′deoxyadenosine; -○-, actinomycin D. *Lower panel:* Poly(A)(−)RNA analyzed on sucrose gradients. Symbols are identical to the upper panel. *Left abscissa:* ^{3}H-activity in cpm per fraction. *Right abscissa:* absorbance. The solid line represents the absorbance at 254 nm with peaks at 4*s*, 18*s* and 28*s* (from left to right)

was studied by analyzing the material which does not bind to the (oligo-dT)-cellulose column and that contains tRNA, rRNA, and poly(A)(−)mRNA. Three homologous pools of bladders were preincubated in the presence of actinomycin D (2 μg/ml) or 3′deoxyadenosine (30 μg/ml) or the diluent and then labeled continuously with ^{3}H-uridine for 180 min. Poly(A)(+)RNA and poly(A)(−)RNA were prepared and analyzed on sucrose density gradients. As shown in Fig. 8, the pattern of inhibition on poly(A)(+)RNA synthesis was strikingly similar for both inhibitors (upper panel) while clearly 3′deoxyadenosine did not inhibit poly(A)(−)RNA to the same extent (hatched area in lower panel) as actinomycin D. Indeed, the RNAs which sediment between 4*s* and 18*s* are likely to represent poly(A)(−)mRNA, were barely inhibited by 3′deoxyadenosine, but were markedly inhibited by actinomycin D (compared to the control) with allowance for the influence of the heavily labeled tRNA peak. tRNA apparently was inhibited to the same extent (~50%) by both drugs. Moreover, the incorporation of ^{14}C methyl group into nuclear ribosomal RNA precursors (which is an index of rRNA synthesis (Rossier *et al.*, 1977*b*) was completely and equally abolished by both inhibitors (data not shown).

Discussion

Our results show that, even in the presence of a marked inhibition of poly(A)(+)RNA (70–80%) by 3′deoxyadenosine, aldosterone can elicit a partial but significant effect on Na^+ transport (Fig. 4). By contrast, actinomycin D at a concentration that produced an equivalent inhibition of poly(A)(+)RNA synthesis (68–75%) completely abolished that response.

The differential effects of the two inhibitors could be related either to their well-characterized effect on RNA metabolism or to some unspecific effects on transport or cell metabolism. Whilst we cannot rule out such effects, there is a great deal of evidence against them: the response of the scc to substrate such as glucose is unimpaired, and the response to vasopressin is intact as shown by Edelman *et al.* (1963) for actinomycin D and by Chu and Edelman (1972) for 3′deoxyadenosine. Based on these assumptions, our results suggest that the difference observed is related to different sites of action of these inhibitors on poly(A)(+)RNA and poly(A)(−)RNA synthesis.

In the present study, actinomycin D and 3′deoxyadenosine inhibited

the synthesis of poly(A)(+)RNA, tRNA, and rRNA to the same extent but not the poly(A)(−)RNA that sedimented between 4*s* and 18*s* which is likely to be poly(A)(−)mRNA. This is consistent with the findings of Breindl and Gallwitz (1974), who showed that 3′deoxyadenosine was unable to inhibit the synthesis of histone mRNA, a well-characterized poly(A)(−)mRNA. We have shown previously that the induction of poly(A)(+)RNA, but not rRNA (Rossier *et al.*, 1977*b*), was of importance in mediating aldosterone-dependent Na^+ transport (Rossier *et al.*, 1974, 1977*a*, 1977*b*; Wilce *et al.*, 1976). The present study suggests that an intact poly(A)(−)mRNA pathway is necessary in order to obtain a complete response[1]. This could be due to the induction of specific poly(A)(−)mRNA by aldosterone or, alternatively, could be related to some permissive effect of this pathway.

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1 It is of interest to note that the steroid ecdysone dependent induction of vitellogenesis in the mosquito is not inhibited by 3′deoxyadenosine, but completely abolished by actinomycin D (Fong & Fuchs, 1976).

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