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ARTERIAL EFFECTS OF ALDOSTERONE AND ANTIMINERALOCORTICOID COMPOUNDS MECHANISM OF ACTION

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Summary—The aim of our work was to study the mechanism of action of aldosterone and antialdosterone compounds on Na^+ and K^+ fluxes in vascular smooth muscle.

In the long term, regulation of salt metabolism depends on aldosterone effects on Na^+, K^+, H^+ and H_2O transport by the renal tubules. Furthermore, it has been shown that aldosterone modifies several epithelial transports, inducing a positive sodium balance [1–4]. The chronic *in vivo* administration of aldosterone modifies transmembrane ionic fluxes in vascular smooth muscle. Garwitz and Jones [5] suggested that aldosterone may enhance net Na^+ transport through the stimulation of the sodium pump.

The results obtained in our laboratory indicate that aldosterone has a direct stimulatory action on ouabain-dependent and on ouabain-independent Na efflux. Furthermore, the mineralocorticoid enhances passive K permeability, as well as the Na pump dependent K influx. Both effects are blocked by antimineralocorticoid compounds. Recent experiments have shown that vasopressin potentiates some of the *in vivo* effects of aldosterone.

ACTION OF ALDOSTERONE AND ANTIMINERALOCORTICOIDS ON TRANSMEMBRANE ^{22}Na EFFLUX FROM ARTERIAL SMOOTH MUSCLE

These experiments were performed on adrenalectomized Sprague–Dawley rats, maintained on 0.9% NaCl in water until they were studied, 7 days after the operation [6].

Ex vivo *effects of aldosterone on* ^{22}Na *efflux*

For the *ex vivo* experiments, the animals were given subcutaneous injections of aldosterone dissolved in a 2.5% ethanol solution. Control adrenalectomized animals were injected with the solvent. The methods used for the study of the transmembrane ^{22}Na efflux have been previously described [7].

Ionic flux experiments on the rat tail artery have shown that aldosterone has a marked effect on transmembrane ^{22}Na efflux. After the subcutaneous injection of 10 μg/kg of aldosterone (Fig. 1), there is a very rapid increase in the ouabain insensitivity ^{22}Na efflux, which starts as early as 15 min after the injection, and is followed by a secondary rise in the passive efflux which attains a plateau 4 h after the mineralocorticoid administration. Under these conditions, aldosterone also increases the ouabain-dependent ^{22}Na efflux; the initial effect being obtained after 1 h and reaching a plateau 3 h later.

These effects appear to be due to an action of aldosterone on mineralocorticoid receptors, since the specific mineralocorticoid antagonist RU28318 and spironolactone (Fig. 3a) blocks the hormone effects on ^{22}Na efflux. Furthermore, the effects of RU26988, a specific glucocorticoid agonist, on ^{22}Na efflux, are negligible even at doses which occupy glucocorticoid-binding sites completely [6]. Our results indicate that aldosterone increases, at doses that determine a renal effect [8], both ouabain-independent and ouabain-dependent ^{22}Na efflux.

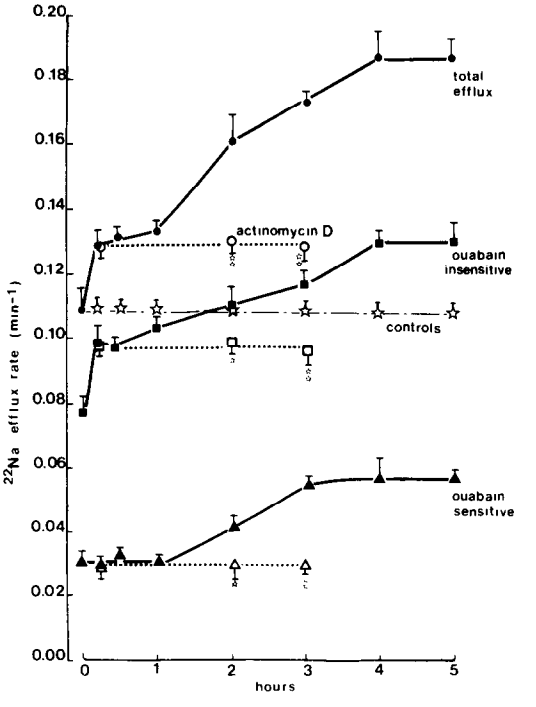

Fig. 1. (Reproduced from [6]). Timing of *in vivo* effects of aldosterone (10 μg/kg) on the ^{22}Na efflux from arterial smooth muscle. ● = total ^{22}Na efflux; ■ = ouabain-insensitive ^{22}Na efflux; ▲ = ouabain-dependent ^{22}Na efflux; ○□△ = the same after pretreating the animals with actinomycin D (50 μg/kg); ☆ = total ^{22}Na efflux after vehicle injection in adrenalectomized controls. The values of ouabain-sensitive and ouabain-insensitive ^{22}Na effluxes after vehicle injection remain stable and are not shown for the sake of clarity. $n = 10$ rats. $^*P < 0.05$. $^{**}P < 0.01$.

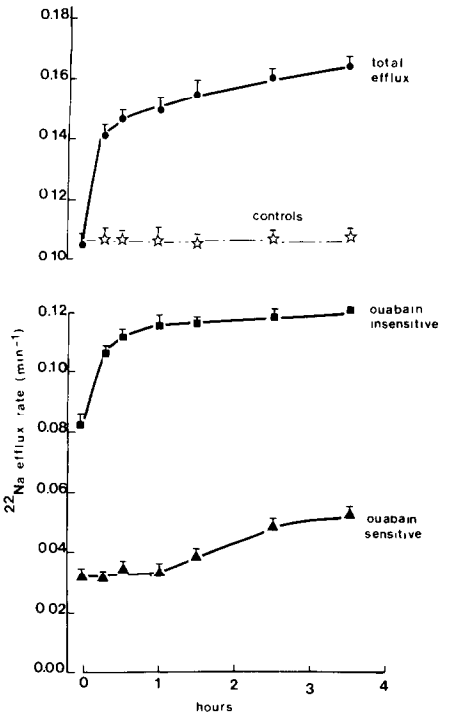

Fig. 2. Modified from [6]). *In vitro* effect of aldosterone (2.10^{-6} M) on ^{22}Na efflux from adrenalectomized rat arterial smooth muscle. ● = total ^{22}Na efflux; ■ = ouabain-insensitive ^{22}Na efflux; ▲ = oubain-dependent ^{22}Na efflux. $n = 10$ rats. $^{*}P < 0.01$. ☆ = total ^{22}Na efflux after exposing the strips to the vehicle (controls).

In vitro *effects of aldosterone*

These experiments were performed by exposing the rat tail artery from adrenalectomized Sprague–Dawley rats to aldosterone. Under *in vitro* conditions (Fig. 2), the mineralocorticoid has the same effects on ouabain-dependent ^{22}Na efflux (sodium pump) as previously shown after *in vivo* administration, namely a late increase giving rise to a plateau phase obtained after 3 h. On the other hand, *in vitro* only the initial rapid action of aldosterone on ouabain insensitive (passive) ^{22}Na efflux was observed, starting 15 min after the exposure to the mineralocorticoid and attaining a plateau 1 h after exposure.

The antimineralocorticoid RU28318 blocks both effects of aldosterone on passive and pump dependent components of the ^{22}Na efflux (Fig. 3b).

Our results indicate that aldosterone has a direct and mineralocorticoid-specific action on the ouabain dependent ^{22}Na efflux from rat tail artery smooth muscle which seems to be identical after *in vitro* exposure and *in vivo* administration of the hormone.

The *in vivo* effects of aldosterone on the ouabain-independent ^{22}Na efflux appear to be due to the addition of two consecutive actions: (1) the initial rapid stimulation, starting as early as 15 min after the administration of the hormone and attaining a plateau at 60 min; (2) and a late rise starting after

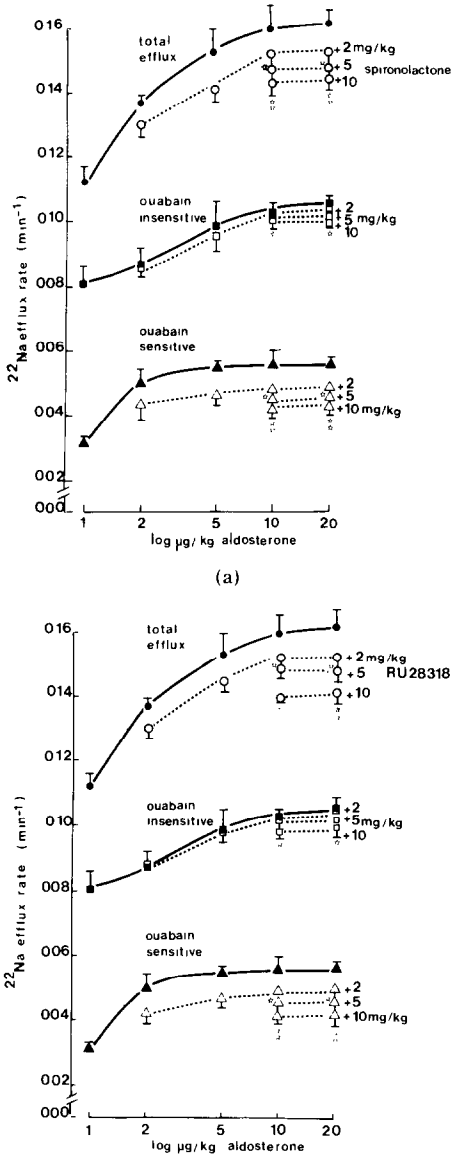

Fig. 3. Modified from [6]). (a) *In vivo* effect of the antimineralocorticoid RU 28 318 on the dose-^{22}Na efflux curves of aldosterone. ● = total ^{22}Na efflux; ■ = ouabain-insensitive ^{22}Na efflux; ▲ = ouabain-dependent ^{22}Na efflux; ○□△ = the same after pretreating the rats with RU 28 318. $n = 10$ rats. $^{*}P < 0.05$. $^{**}P < 0.01$. (b) *In vitro* effect of the antimineralocorticoid RU 28 318 on the dose-^{22}Na efflux curves of aldosterone. ● = total ^{22}Na efflux; ■ = ouabain-insensitive ^{22}Na efflux; ▲ = ouabain-dependent ^{22}Na efflux; ○□△ = the same after pretreating the rats with RU 28 318. $n = 10$ rats. $^{*}P < 0.05$. $^{**}P < 0.01$.

60 min, reaching a new plateau 4 h after the injection. Both the initial and the late stimulations of passive Na efflux are due to an action on aldosterone receptors, but only the early rapid effect appears to be the result of a direct action of the hormone on vascular smooth muscle. Indeed, the late increase in passive (ouabain-insensitive) ^{22}Na efflux does not

exist after *in vitro* exposure to aldosterone, thus suggesting that part of the response is secondary to the intervention of a humoral factor.

INTERACTIONS BETWEEN ALDOSTERONE AND VASOPRESSIN

Vasopressing might be the humoral factor mediating some of the *in vivo* effects of aldosterone on vascular smooth muscle. Indeed, DOCA and salt hypertension does not develop in vasopressin deficient animals neither in Brattleboro rats [9, 10] nor after the lesion of the anteroventral region of the third ventricle [11] in Wistar rats. Vasopressin appears to play a role in the regulation of blood pressure, interacting with other vasoactive mediators and hormones. The injection of a vasopressin antiserum in rats with a malignant or a benign DOCA and salt hypertension induces a transient fall of blood pressure to normal or subnormal levels [12]. Vasopressin has constrictor effects. *In vitro*, the peptide contracts microscopic and large blood vessels and potentiates the vascular smooth muscle responses to other vasoconstrictors like catecholamines and angiotensin II [13].

In vivo *effects of aldosterone on* ^{22}Na *efflux. Antagonism by* $d(CH_2)_5Tyr(Me)AVp$

Aldosterone was injected (10 μg/kg s.c.) to adrenalectomized Sprague–Dawley rats (7 days), in the presence of $d(CH_2)_5$Tyr(Me)AVp. This peptide is an antagonist of the vascular actions of vasopressin, synthetized by Manning *et al.*[14]. The antagonist was infused to conscious, unrestrained rats at a rate sufficient to block the hypertensive effects of vasopressin [15]. Under these conditions, (Fig. 4) aldosterone only induces the early stimulation on the passive ^{22}Na efflux, the secondary rise of the ouabain-independent ^{22}Na efflux being suppressed by the antagonist. Similarly, even if the kinetics of the action of the mineralocorticoid on the ouabain-dependent ^{22}Na efflux does not appear to be modified, the magnitude of the plateau rise of this component seems to be lower than the response observed in the absence of the vasopressin antagonist (see Fig. 1).

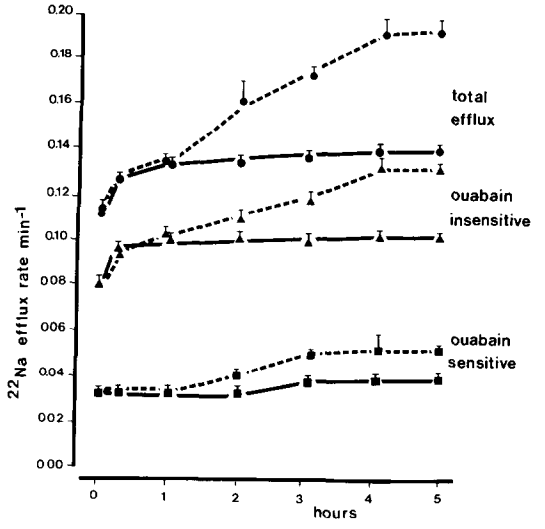

Fig. 4. *In vivo* effects of aldosterone in the presence of the antagonist $d(CH_2)_5$ Tyr(Me)AVp. ●---● = total ^{22}Na efflux in the absence of the antagonist; ●—● = total ^{22}Na efflux in the presence of the antagonist; ▲---▲ = ouabain-insensitive ^{22}Na efflux in the absence of the antagonist; ▲—▲ = the same in the presence of the antagonist; ■--- ■ = ouabain-sensitive ^{22}Na efflux in the absence of the antagonist; ■—■ = ouabain-sensitive ^{22}Na efflux in the presence of the antagonist.

In vivo *effects of aldosterone on* ^{22}Na *efflux from Brattleboro rats*

Experiments were performed on adrenalectomized animals. The rats were maintained on 0.9% NaCl in water until they were studied 7 days after surgery. The injection of 10 μg/kg of aldosterone s.c. to these animals induces changes in ^{22}Na efflux from the rat tail artery (Fig. 5), which are practically identical to those observed either *in vivo* in vasopressin antagonist treated Sprague–Dawley rats or *in vitro* in tail arteries of the same strain. Namely, the late increase in the passive ^{22}Na efflux is absent.

Effects of aldosterone on plasma levels of vasopressin

The previous results strongly suggested that: (1) the presence of vasopressin is needed in order to induce the secondary rise in ^{22}Na efflux; (2) vasopressin might be released by aldosterone.

Experiments performed in adrenalectomized Sprague–Dawley rats have shown that after the s.c. injection of 10 μg/kg of aldosterone there is a significant rise in circulating levels of vasopressin, which was detectable 2 h after the administration of the mineralocorticoid. The peak was observed 4 h after the injection [16].

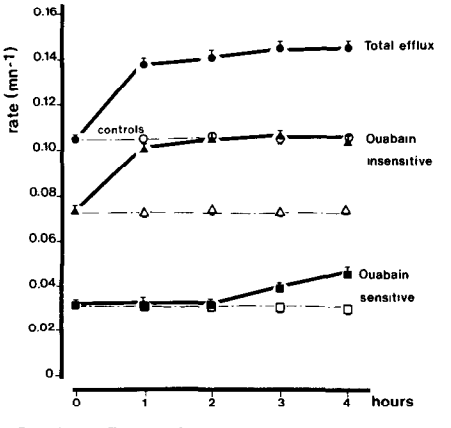

Fig. 5. *In vivo* effects of aldosterone (10 μg/kg) on the ^{22}Na efflux from the adrenalectomized Brattelboro rat tail artery. ● = total ^{22}Na efflux; ▲ = ouabain-insensitive ^{22}Na efflux; ■ = ouabain-sensitive ^{22}Na efflux. ○; △; and □ = the same in vehicle injected animals.

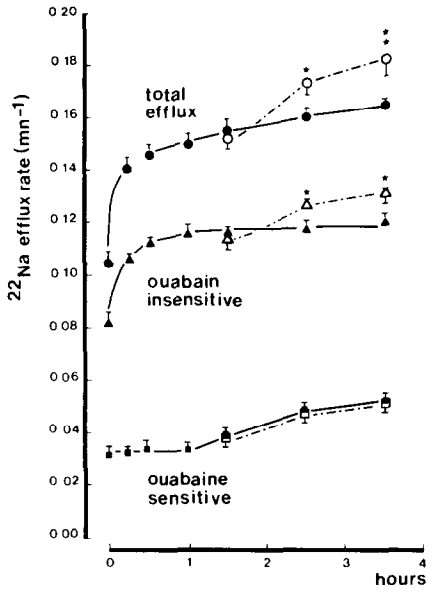

Fig. 6. (Modified from [15]). Comparison of the *in vitro* effects of aldosterone alone (solid lines) and associated to vasopressin (broken line) on ^{22}Na efflux, $n = 8$ rats.

Synergy of action of aldosterone and vasopressin on ^{22}Na efflux

Vasopressin at the concentration of 10^{-11} M compatible with physiological levels [17], applied *in vitro* to tail arteries from adrenalectomized Sprague–Dawley rats (7 days), induces a late increase in passive ^{22}Na efflux, a plateau being attained after 3 h. Simultaneously, the ouabain-dependent ^{22}Na efflux was stimulated, the maximal effect being observed after 3 h of exposure to the peptide [15].

When aldosterone and vasopressin are added at the same time to the incubation medium the overall effect obtained mimics the *in vivo* effects of aldosterone (Fig. 6). These findings together with the above mentioned results of increase in plasma levels of vasopressin induced by the injection of aldosterone, suggest strongly that the *in vivo* effects of aldosterone on ^{22}Na efflux result from the addition of the direct stimulation of passive sodium permeability as well as of the sodium pump through a direct activation of mineralocorticoid receptor in arterial smooth muscle and the potentiation of aldosterone effects by vasopressin which results in a secondary rise in passive ^{22}Na efflux.

ACTION OF ALDOSTERONE AND VASOPRESSIN ON ^{86}Rb FLUXES

^{86}Rb was used as a marker of K^+ movements. We have shown previously that this isotope is well adapted for a correct evaluation of transmembrane K^+ fluxes in vascular smooth muscle [18]. When injected s.c. at the dose of 10 μg/kg to adrenalectomized Sprague–Dawley rats, aldosterone induces an increase in ^{86}Rb efflux from tail arteries. A plateau effect was observed 3 h after the injection. This action of aldosterone is reduced in animals treated with the vasopressin antagonist $(dCH_2)_5$Tyr(Me)AVp used in the above mentioned conditions [16].

The *in vitro* effects of aldosterone (10^{-8} M) are lower than the effects induced *in vivo* on ^{86}Rb efflux. The simultaneous addition of 10^{-8} M and 10^{-11} M vasopressin has a potentiating action on ^{86}Rb efflux from the tail artery from adrenalectomized Sprague–Dawley rats.

CONCLUSION

The results of our laboratory indicate that aldosterone increases at doses [8] and concentrations [19] compatible with a physiological effect, both ouabain dependent and Na pump dependent ^{22}Na fluxes, as well as the membrane permeability to K^+ in arterial smooth muscle.

These aldosterone effects appear to be mineralocorticoid dependent since they are blocked dose-dependently by the antimineralocorticoid compounds spironolactone and RU28318. The rat tail artery contains mineralocorticoid as well as glucocorticoid receptors (Bouton, unpublished data). Nevertheless, the specific glucocorticoid receptor agonist RU26988 has negligible effects at doses that saturate glucocorticoid receptors [20]. Furthermore, it has been shown that mineralocorticoid receptors are present in rabbit aorta, femoral and carotid arteries, as well as in lamb and rabbit brain small arteries [19].

Our results indicate that aldosterone has a direct and mineralocorticoid-specific action on ouabain-dependent ^{22}Na efflux from rat tail artery smooth muscle, which appears to be identical after *in vivo* administration and *in vitro* exposure to the hormone.

In experimental conditions, aldosterone stimulating effects appear after a long delay and are suppressed by actinomycin D [6]. This suggests that this mineralocorticoid action follows the usually proposed mechanism involving the transcription of genomic information [21].

The early (direct) effect of aldosterone on ouabain-independent ^{22}Na efflux has a rapid onset—less than 15 min—hardly compatible with the time necessary for the activation of the target cell genoma. Indeed, this action is not blocked by actinomycin D [6]. Whatever the mechanism, the early *in vitro* effects of aldosterone may be due to an action of the mineralocorticoid on receptors having characteristics close to cytosolic binding sites. Indeed, the antagonists RU28318 and spironalactone early and late dose–response curve are identical, thus showing that they have similar pharmacological properties.

We have considered the possibility that the late *in vivo* effects of aldosterone on ouabain-independent ^{22}Na efflux and ^{86}Rb efflux could be explained by the action of a humoral factor. Our recent results indicate that vasopressin released by aldosterone can

explain the actions of the mineralocorticoid on passive Na^+ and K^+ movements, observed 2 h after injection.

The direct and indirect effects of aldosterone on the transmembrane Na and K transport in arterial smooth muscle may be important for the regulation of free intracellular sodium. It may be suggested that, as a consequence of this direct and vasopressin mediated effects on smooth muscle, aldosterone participates in the regulation of vascular tonus and blood pressure. Nevertheless, further experiments are needed to determine the effects of aldosterone on smooth muscle contraction.

The antihypertensive effect of antimineralocorticoid compounds is correlated with their diuretic action. Our findings suggest that the vascular smooth muscle, and possibly other extrarenal tissues can also be a target for aldosterone antagonists.

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