dynamic pertubations by acting through the renin-angiotensin system. It has been repeatedly shown that increased renal sympathetic tone and/or decreased renal blood flow are powerful stimuli for increased renal renin production[4,8]. Due to the long half life of renin (recent estimates range between 2 and 4 h) even occasional, transient 'puffs' of renin could summate to chronically elevate plasma or central nervous system (CNS) levels of angiotensin II. An elevated level of angiotensin II could, in turn, increase arterial pressure by its direct constrictor effect on smooth muscle[9], by its pressor effects on the CNS[10], or by potentiation of the release of catecholamines[11,12].

The results of this study suggest a mechanism whereby even occasional aversive stimuli might become associated with discrete environmental cues; then, the mere existence of the cues, only occasionally reinforced with the aversive stimuli, could elicit the complete constellation of cardiovascular and humoral responses.

Résumé. Des chiens ont été conditionnés à attendre un choc électrique 10 sec après le début d'un stimulus auditif. Ils ont présenté une considérable vasoconstriction rénale, transitoire et d'origine nerveuse, faisant partie de la réponse conditionnée cardiovasculaire.

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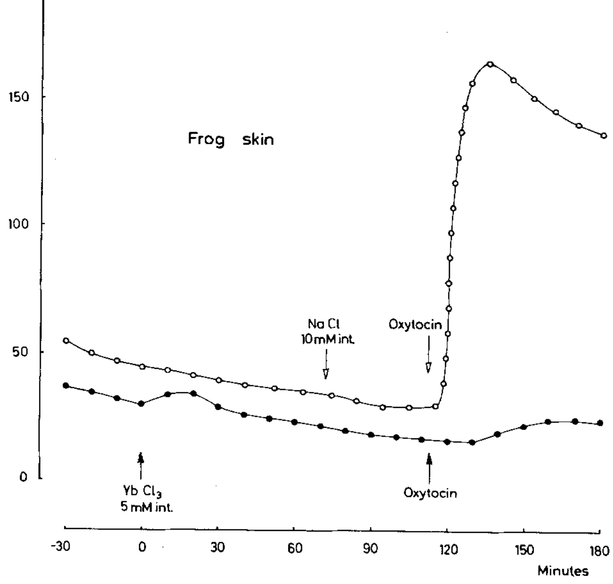
Lanthanides and Amphibian Epithelia: Block of the Hormone-Induced Stimulation of Sodium and Water Transport

Previous work from this laboratory has shown that lanthanides increase the permeability to sodium of frog skin when added to the external surface of the epithelium[1]. An interaction between La^{3+} and Ca^{2+}, predicted by Lettvin et al.[2] and verified in many biological systems[3], is the most likely explanation of the changes in permeability observed in frog skin[1]. A large body of evidence suggests that Ca^{2+} plays a key role in the cell processes triggered by hormone action[4]. It thus seemed justified to investigate if lanthanides alter the stimulus-effect coupling of hormones active on amphibian epithelia.



The results reported here do show that lanthanides, added to the medium bathing the internal surface of frog skin, block the stimulation of sodium and water transport induced by oxytocin and norepinephrine.

The bulk of our studies on sodium transport was performed with the ventral skin of frogs *Rana ridibunda*. Standard techniques were used to measure short circuit current (SCC), taken as a measure of net sodium flux[5]. Since both basal water flows and hydrosmotic responses to hormones are quite variable in frog skin, the skin of toads, *Bufo bufo*, was used in the water flow studies. Water flow measurements were performed with an automatic, optical technique, in which the movement of the meniscus inside a pipette attached to the flow chamber is followed continuously[6].

Addition of La^{3+} (5 mM) to the internal side of frog skin resulted in a marked inhibition of the increase in SCC elicited by supramaximal concentrations of oxytocin. In a series of 21 paired experiments, the average increment

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Fig. 1. Inhibition of the natriferic effect of oxytocin (50 mU/ml) by Yb^{3+} added to the solution bathing the internal surface of frog skin. Experiments were performed with a lucite double chamber filled with aerated *Tris*-Ringer solutions. SCC, short circuit current. ○—○, control tissue; ●—●, tissue exposed to Yb^{3+}. To rule out an osmotic effect of the lanthanide solution on SCC, the concentration of NaCl in the internal medium bathing the control tissue was raised by 10 mM.

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in SCC induced by oxytocin in control skins was 25.2 ± 2.76 µA cm^{-2} (average ± SEM), while in the skins exposed to La^{3+} the increment was 11.2 ± 1.98 µA cm^{-2} ($p < 0.001$). Similar results were obtained with much lower concentrations of La^{3+} (1 mM), average increments in SCC being respectively 29.0 ± 5.82 and 10.7 ± 3.62 µA cm^{-2} ($N = 5$, $p < 0.01$). Additional experiments were performed with the skin of toads, *Bufo bufo*, in order to compare both the natriferic and the hydrosmotic effects of hormones in the same tissue. A similar inhibition of the natriferic effect of oxytocin was observed in toad skins exposed to La^{3+} ($N = 10$, $p < 0.001$). Analogous results were obtained with other hormones (norepinephrine, arginine-vasopressin), other lanthanides (Ce^{3+}, Sm^{3+}, Yb^{3+}) and other epithelia (the bladder and the skin of toads *Bufo marinus*). Figure 1 depicts the time course of an experiment in which the rise in SCC induced by oxytocin was almost totally blocked after exposure of frog skin to a heavy lanthanide (Yb^{3+}).

Effects of La^{3+} (5 mM) on the hydrosmotic action of several agents

	N	Δ J_{H_2O} µl min^{-1} cm^{-2} (average ± SEM)	P
Oxytocin (50 mU/ml)	11	0.48 ± 0.067	< 0.001
La^{3+} + oxytocin		0.06 ± 0.019	
Norepinephrine (6×10^{-8} M)	10	0.55 ± 0.082	< 0.01
La^{3+} + norepinephrine		0.13 ± 0.060	
cAMP (5 mM)	7	0.39 ± 0.063	< 0.005
La^{3+} + cAMP		0.19 ± 0.034	
Theophylline (10 mM)	16	0.32 ± 0.026	> 0.95
La^{3+} + theophylline		0.32 ± 0.036	

N, number of paired studies. Δ J_{H_2O}-increment in water flux, calculated as the difference between the steady state value before the agent was added and the peak value obtained during the hydrosmotic response.

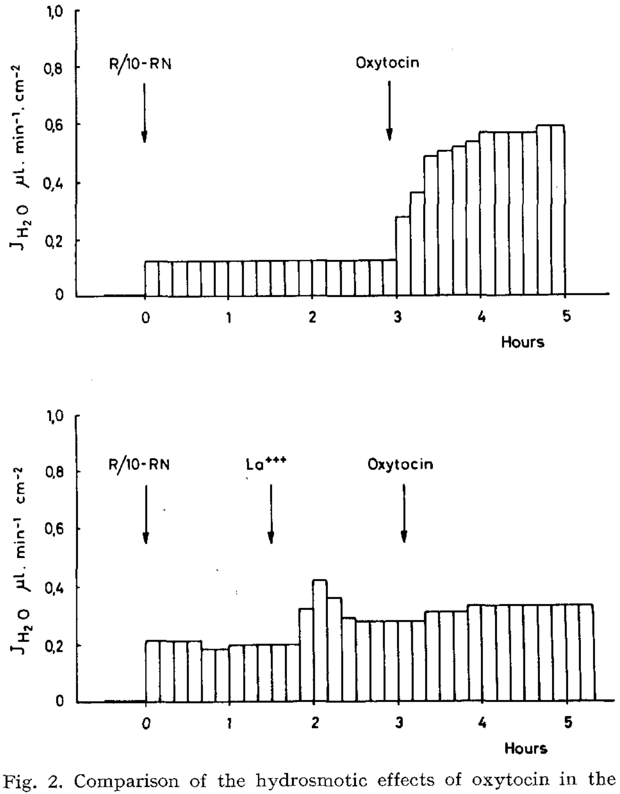

Fig. 2. Comparison of the hydrosmotic effects of oxytocin in the absence and in the presence of La^{3+} (LaCl$_3$, 5 mM), in 2 pieces of the same toad skin. J_{H_2O}-transepithelial water flux, calculated from the continuous recording of the displacement of the meniscus in a horizontal pipette (6). R/10-RN transepithelial osmotic gradient: RN, normal *Tris*-Ringer solution, bathing the internal side of the skin; R/10, the same solution diluted 10 times, bathing the external side.

It is generally acknowledged that neurohypophyseal hormones and norepinephrine stimulate sodium transport in frog skin by activating the adenylate cyclase of the epithelial cells[7–9]. Several sites along the stimulus-effect coupling of oxytocin and norepinephrine could be considered as possible targets of lanthanides, to explain the block of the hormone action. In order to explore some of these possibilities, two other interactions were studied: a) between La^{3+} and cyclic AMP (cAMP); b) between La^{3+} and theophylline.

Experiments with cAMP (5 mM) showed an inhibition of the natriferic effect of the nucleotide. In 11 paired experiments with frog skin, the average increase in SCC elicited with cAMP was 16.1 ± 2.15 µA cm^{-2}, while, in the presence of La^{3+}, the increase was only 5.6 ± 1.21 µA cm^{-2} ($p < 0.005$). Finally, and in contrast with the results reported so far, the natriferic effect of theophylline (10 mM) was not modified by La^{3+}. Statistical analyses of 14 paired experiments showed no significant difference between the results obtained both in the absence, and in the presence, of La^{3+}, the values being respectively 20.0 ± 2.48 and 18.5 ± 2.83 µA cm^{-2} ($p > 0.50$).

It is well known that neurohypophyseal hormones and norepinephrine stimulate not only sodium transport but also water transport across amphibian epithelia[7–9]. We investigated therefore the effects of lanthanides on the increase in water flux induced by these hormones to test whether similar steps of the stimulus-effect coupling were affected by the trivalent ions.

The water flux studies were designed according to the same experimental framework previously used in the sodium transport studies. The results are summarized in the Table. The effects of lanthanides on the hydrosmotic flow show a striking parallelism with those previously found with sodium transport. In both instances there is a significant inhibition of the action of oxytocin, norepinephrine and cAMP, while the effect of theophylline is not modified (Table). The block of the hormone is often almost complete, either with oxytocin (Figure 2) or with norepinephrine (Figure 3). A slight and transient stimulation of water transport can also be observed immediately after the addition of the lanthanide (Figures 2 and 3). It is interesting that a similar effect was found with SCC (Figure 1), the stimulation being followed most often by an inhibition of SCC.

The lanthanide-induced inhibition of both the natriferic and the hydrosmotic effects of neurohypophyseal hormones, norepinephrine and cAMP suggests a block in the hormonal stimulus-effect coupling beyond the generation of cAMP. However, a concomitant effect in the early phase of this coupling, i.e., between the receptor occupancy by the hormone and the activation of adenylate cyclase, cannot be excluded. It is also possible that the block of cAMP might be due to a decrease in the permeability of the epithelial cells to the nucleotide, in the presence of La^{3+}.

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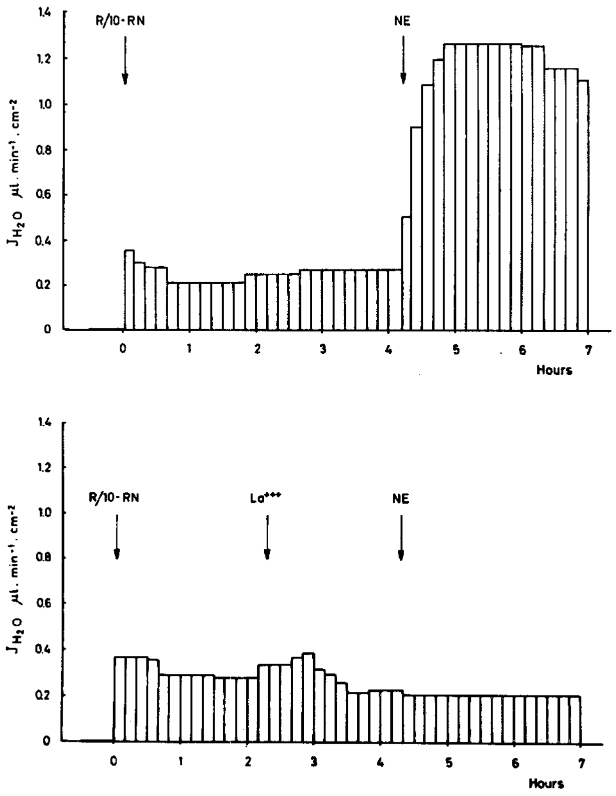

Fig. 3. Comparison of the hydrosmotic effects of norepinephrine (NE, 6×10^{-8} *M*) on 2 pieces of the same toad skin, in the absence, and in the presence, of La^{+++} ($LaCl_3$, 5 m*M*). Technique as described in Figure 2. Note the transient stimulation of water flow by La^{3+} and the sustained block of the hormone action.

In view of the strong interaction between Ca^{2+} and La^{3+} observed in many biological systems[3], it is reasonable to assume that lanthanides affect Ca^{2+} influx or some other critical calcium-dependent step in the hormonal stimulus-effect coupling[4]. The results observed with theophylline do not formally contradict such a hypothesis. There is increasing evidence that theophylline does more on frog skin than simply inhibit phosphodiesterase[10,11], and, in pancreas, an effect of theophylline on the translocation of intracellular Ca^{2+} has been postulated[12,13]. In addition, our data are compatible with those reported by WEISS[14] in frog skeletal muscle, which showed that La^{3+} did not inhibit the effects of caffein on muscle, in particular those concerning calcium movements.

Further studies are necessary to elucidate the site and mode of action of lanthanides in amphibian epithelia. It is apparent, however, that lanthanides are useful tools for studying the mode of action of hormones and the role of calcium in such biological processes[15,16].

Résumé. L'addition de lanthanides au milieu baignant la surface interne (séreuse) des épithélia d'amphibien provoque une inhibition de l'effet hydrosmotique et natriférique de l'ocytocine, de la nor-épinéphrine et de l'AMP cyclique. Le rôle d'une probable interaction calcium/lanthanides est discuté.

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Effect of Temperature and Concentration on the Anomalous Potassium Uptake of Thermophilic Plants

Experimental studies of temperature effects on the ion uptake (IU) of plants are being carried out in several laboratories. The present general opinion is that the intensity of IU by plants increases considerably on the rise of temperature[1–3]. Contrary to this, it was established that an anomalous initial K^+ uptake (AIPU) and an anomalous K^+ efflux occurred in the roots of certain thermophilic plants at chilling temperatures[4]. No departure from the regular IU was revealed for NH_4^+ and anions[5,6]. The anomaly in the K^+ uptake (PU) was diminished at lower pH, while no or very small changes could be observed in the AIPU if uncouplers were employed[7,8]. In this short paper, some properties of the AIPU of low-salt plant roots will be briefly discussed.

Material and method. Cucumber (*Cucumis sativus* cultivar Csemege fürtös) seedlings were grown in 5×10^{-4} *M* $CaSO_4$ solution under well-controlled conditions as described earlier[6]. 6–7 cm long roots of 7-day-old plants were excised and washed in distilled water for 10 min at room temperature. About 3 g of the root material was placed in 500 ml aerated, isotopically-labelled uptake solutions kept at different temperatures between 0 and 21 °C, for given periods of time. The temperature of the absorption solution was constant within 1 °C during the incubation process. The pH of the uptake solution was adjusted to 6.4–6.5. No noticeable change in pH occurred during the experiments. For technical reasons, ^{86}Rb was

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