Plasma DBH activity, urinary catecholamine excretion, blood pressure and pulse rate before and during insulin-induced hypoglycemia

Insulin (U/kg)	Plasma DBH activity (μmoles/min/l plasma)		Adrenalin (μg/3 h)		Noradrenalin (μg/3 h)		Pulse rate (min)		Blood pressure (mm Hg)	
	Before insulin	After insulin[a]	Before insulin	After insulin	Before insulin	After insulin	Before insulin	After insulin[a]	Before insulin	After insulin[a]
0.1	22.4 ± 3.1[b] (5)	23.6 ± 2.8	2.7 ± 0.1	12.7 ± 1.7[e]	11.9 ± 1.5	10.9 ± 1.2	60.2 ± 3.2	72.4 ± 3.5[c]	129.2 ± 4.5	144.8 ± 5.7
0.15	16.8 ± 1.6 (6)	26.5 ± 1.3[e]	2.2 ± 0.3	12.3 ± 0.5[e]	10.2 ± 1.6	11.9 ± 1.7	53.3 ± 3.3	70.7 ± 5.8[c]	110.0 ± 1.2	137.0 ± 7.6[d]

[a] Highest level obtained during hypoglycemia. [b] Figures are given as mean ± standard error with number of specimens shown in parenthesis. The statistical significance of differences between the mean values of before and after insulin administration was certified by Student's *t*-test. [c] $p < 0.05$; [d] $p < 0.01$; [e] $p < 0.001$.

with a larger dose of insulin, may suggest that the adrenal glands react more promptly and extensively to a milder stress than do the sympathetic nerves.

The present data indicate a close correlation between the plasma DBH activity and the sympathetic nervous function during severe stress. The estimation of plasma DBH activity is highly useful in research and clinical practice to assess the sympathetic nervous activity, since it is of simpler procedure, less time-consuming and yields more stable and accurate values than the determination of plasma or urinary noradrenalin.

Résumé. Nous avons étudié l'activité de la dopamine-β-hydroxylase du plasma humain. Elle a été remarquablement accrue par l'administration de grandes quantités d'insuline. Les corrélations entre cette activité et celle des systèmes nerveux sympathiques sont également discutées.

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Renal Blood Flow Changes During Aversive Conditioning in the Dog

In man and other animals, many aversive stimuli which elicit arterial hypertension also increase renal vascular resistance[1–3]. These and other observations[4] support the hypothesis that a causal relationship may exist between emotion-related renal vasoconstriction and hypertension. The frequency of occurrence of discrete aversive stimuli, under normal environmental conditions, might appear to be too low to account for sustained or frequent decreases in renal blood flow. An etiological mechanism for arterial hypertension might exist, however, if previously neutral, environmental cues became associated with aversive stimuli so that occurrence of either the aversive stimulus or the associated cues elicited renal vasoconstriction.

The results of the present study indicate that a previously neutral environmental cue, once associated with an aversive stimulus (using classical conditioning techniques) can, by itself, lead to neurally mediated renal vasoconstrictions.

Each of four adult, male mongrel dogs (12–16 kg) underwent several 1-hour habituation sessions in a restraint harness located within a specially-designed experimental chamber[5]. Then, during aseptic surgery, an electromagnetic flow transducer of appropriate diameter and an inflatable occlusion cuff (for determining zero blood flow) were fitted around the left renal artery. Care was taken to avoid injury to the renal nerves. In addition, an 18 gauge polyvinyl catheter was inserted into the aorta via a carotid artery. After recovery from surgery and stabilization of flow baselines (5 to 10 days) each dog was again placed in the chamber where continuous records of renal blood flow and arterial pressure were recorded.

The orienting reflex to the nonsystematic presentation of a 10-sec tone was habituated. Then each dog was exposed to a series of Pavlovian conditioning trials. A 10-sec tone (CS) was paired at termination with the occurrence of an unavoidable electric shock of 3–6 mA intensity and 0.5 sec duration (US) presented by a constant current source to the hind legs. 10 pairings were made during a 40 min period. Levels of mean arterial pressure, mean renal blood flow and renal vascular resistance were calculated from the polygraph tracings for each 2-sec interval beginning 6 sec before the CS onset and ending 10 sec after US onset.

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Panel A of the Figure illustrates a representative cardiovascular record during the 5th pairing of tone and shock in 1 dog. Within 2 sec of the onset of the CS, renal blood flow began to decrease and arterial pressure began to rise. 8 sec after the onset of the CS the peak renal vascular resistance of 215% of control was attained. The maximum level of resistance was not sustained. 6 sec after presentation of the shock, renal resistance had returned to within 17% of control.

Panel B of the Figure illustrates the averaged conditioned responses for 4 dogs during aversive conditioning trials 6–10. Renal blood flow decreased and arterial pressure increased within 2 sec of the onset of the CS. Maximum resistance was attained within 10 sec. 4 sec after presentation of the shock, renal resistance had begun to fall. However, renal resistance often remained elevated by 15% for as long as 1 min after the US.

The results of this study show that a striking element of the *conditioned* response to aversive stimuli in dogs is a prompt decrease in renal blood flow accompanied by an increase in arterial pressure. Thus, renal vasoconstriction can be elicited not only by aversive stimuli but by discrete environmental cues (CS's) which suggest potential occurrences of aversive stimuli. The results confirm earlier studies in man and other animals which suggested that anxiety-producing environments resulted in decreased renal blood flow[1–3]. In these earlier behavioral studies, however, the necessary use of indirect measurements of renal blood flow precluded an accurate evaluation of either the magnitude or time-course of flow changes. One recent study using electromagnetic flowmeters[6] has demonstrated only *unconditioned* renal vasoconstrictions in dogs subjected to noxious stimuli. These vasoconstrictions resembled those observed in the present study in both amplitude and time course.

The continuous recordings of renal blood flow obtained in the present study permit the following observations. First, the rapid onset (2 sec onset and 10 sec peak latency) of conditioned renal vasoconstriction following presentation of the CS suggests that the earliest and largest vasoconstrictions were neurally mediated. The more prolonged component of the conditioned renal vasoconstrictions may have been mediated by a combination of increased renal sympathetic tone and circulating humoral agents. Second, the striking resemblence between the conditioned cardiovascular responses and responses elicited by electrical stimulation of hypothalamic or mesencephalic regions which organize 'defense' behavior[7], suggests that the environmental cues (CS's) may have acted by mobilizing the classical 'defense response'. The short duration of the neurogenic renal vasoconstriction may have been related to the short duration of the CS (10 sec). However, renal vasoconstrictions and increases in renal nerve activity elicited by prolonged 'defense area' stimulation exhibit a similarly short duration although several other cardiovascular components of 'defense' behavior exhibit sustained responses to stimulation[7].

It is unlikely that unconditioned, neurogenic vasoconstrictions could, in themselves, play any long-term hemodynamic role in the etiology of hypertension. Yet even transient increases in renal sympathetic tone or in renal vascular resistance can elicit prolonged hemo-

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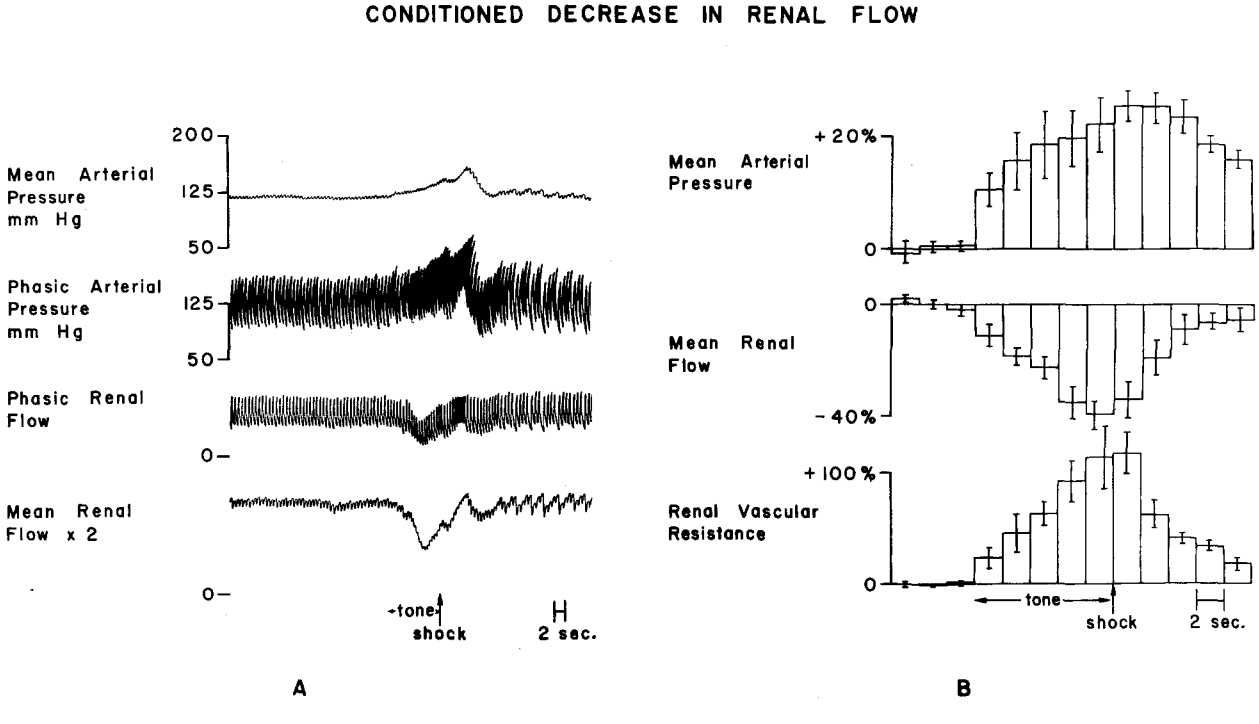

Panel A) physiological record of the response of a dog to the 5th pairing of a 10 sec tone with a shock to the leg. Panel B) mean changes (± SE) in renal arterial pressure, flow and vascular resistance elicited by the 6th through 10th pairings of tone and shock in 4 dogs.

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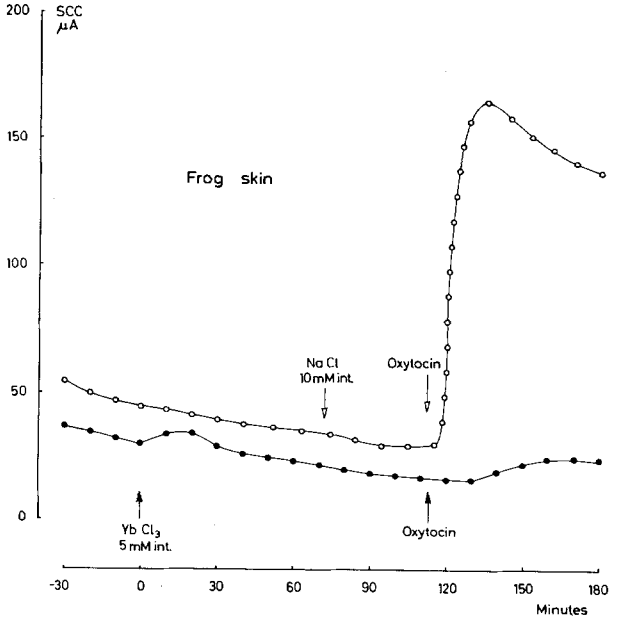
Lanthanides and Amphibian Epithelia: Block of the Hormone-Induced Stimulation of Sodium and Water Transport

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 m*M*) 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



← 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 m*M*.

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