Effects of a Synthetic Vasopressin on Na^+, K^+, and H^+ Exchanges in the Rat.* (30498)

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The action of vasoconstrictor and vasodilator substances is associated with dynamic changes of sodium and potassium between cells and environment in blood vessels(1). Evidence for these exchanges has been obtained by intermittent or continuous monitoring of blood following administration of vasoactive agents(2) and by direct chemical analysis of arterial blood samples(3). In this light, we have now examined the acute effects in the rat of a synthetic vasopressin (PLV-2, Sandoz) and of a synthetic oxytocin (Syntocinon, Sandoz) using both continuous monitoring of arterial blood with glass electrodes and chemical analysis of aorta samples to define the pattern of ion redistribution. Since recent studies have indicated that ion binding may be an important feature of ion partition in vascular smooth muscle(4) we have extended our methods to monitor all of the involved monovalent cations, that is, Na^+, K^+, and H^+, simultaneously.

Methods. Adult male albino rats of an inbred Wistar strain weighing 300 g or more were used throughout. They were anesthetized with a pentobarbitone-phenobarbitone combination as previously described(5).

For the studies involving chemical analysis of tissue, standard methods as previously described were used(5). The procedure involves ligation of the renal pedicles followed by intravenous injection of a carefully measured amount of inulin. Following a period of 3 hours for equilibration a sample from the midsection of the gastrocnemius muscle and a length of aorta were excised as rapidly as possible. The samples were quickly dissected free of connective tissue and placed in preweighed bottles for immediate weighing. Inulin, Na, and K in the tissues and in a sample of arterial blood collected in the terminal minute were analyzed by conventional methods modified for the small samples available.

For the studies involving continuous monitoring of blood ion activity a systemic arterial stream was exteriorized by connecting polyethylene tubing from the carotid artery of one side to the corresponding femoral vein and flow was regulated with a miniature constant volume pump. Flow-through type Na^+, K^+, and H^+ sensitive glass electrodes were interposed in the stream beyond the pump. Blood pressure was monitored with a pressure transducer (Statham) connected to the femoral artery.

The Na^+ and K^+ electrodes with a single calomel reference were connected to a pair of Vibron 33B electrometers (Electronic Instruments Ltd.) and from these to a transistorized unit, "Texada," for continuous linearization and computation(6). The final result expressed in mEq/l was inscribed with a Grass Model 5 Polygraph. The H^+ electrode and its own calomel reference were connected separately to a Cary Model 31 electrometer (Applied Physics Corp.) and from this inscribed directly in millivolts previously calibrated for conversion to pH units.

Results. Distribution of Na, K, and water in skeletal muscle and in aorta following PLV-2. The effect of PLV-2 on the partition of Na, K, and water in skeletal muscle and in aorta was studied in 2 separate experiments. In both experiments, a threshold dose of 2 mU of PLV-2 in 0.1 ml saline was given subcutaneously to 8 inulin-loaded, nephrectomized rats and the saline alone to a matched group of controls. Tissues were excised 15 minutes after injection. The results are presented in Table I.

Even though a threshold dose was used in order to avoid unphysiological effects, 3 changes occurred consistently. First, the extracellular fluid volume in both skeletal muscle and aorta was reduced in the groups receiving PLV-2 compared to their respective controls. Second, there was an increase in the

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TABLE I. Ion Partition in Skeletal Muscle and in Aorta 15 Minutes After Subcutaneous Administration of 2 mU of PLV-2.

	Exp 1		Exp 2	
	Control	PLV-2, 2 mU	Control	PLV-2, 2 mU
Muscle (gastrocnemius)				
Total H_2O, ml/kg	754.7 ± .7	754.0 ± .8	758.1 ± .7	757.8 ± 1.0
E.C.F. (inulin), ml/kg	66.2 ± 1.7 Δ	63.8 ± 1.0	58.1 ± 2.3 Δ	57.7 ± 1.4
Total Na, mEq/kg	17.2 ± .1	16.8 ± .1	16.4 ± .2	17.2 ± .1
Na_i, mEq/kg	8.1 ± .2 Δ	8.7 ± .4	8.2 ± .4 Δ	9.2 ± .2†
$[Na]_i$, mEq/l	11.7 ± .3 Δ	12.5 ± .5	11.7 ± .5 Δ	13.1 ± .3†
Total K, mEq/kg	115.8 ± .3	115.7 ± 1.1	116.4 ± .8	115.9 ± .5
K_i, mEq/kg	115.5 ± .3	115.4 ± 1.1	116.1 ± .8	115.6 ± .5
$[K]_i$, mEq/l	167.9 ± .5	166.1 ± 1.8	165.9 ± 1.3	165.1 ± .9
No. of animals	8	8	8	8
Aorta				
Total H_2O, ml/kg	672.6 ± 3.6	667.8 ± 6.6	649.2 ± 4.8	640.1 ± 6.5
E.C.F. (inulin), ml/kg	327.6 ±11.2 Δ	315.2 ±15.8	310.9 ± 7.7 Δ	304.3 ±12.0
Total Na, mEq/kg	77.3 ± 1.0	80.0 ± .7	86.2 ± 1.1	89.0 ± .8
Na_i, mEq/kg	31.7 ± 1.2 Δ	37.5 ± 1.8*	41.9 ± 1.2 Δ	46.3 ± 2.0
$[Na]_i$, mEq/l	91.8 ± 1.3 Δ	102.3 ± 2.0*	124.2 ± 3.3 Δ	138.0 ± 4.8*
Total K, mEq/kg	39.9 ± .6	41.6 ± .3	47.1 ± .6	45.9 ± .9
K_i, mEq/kg	39.3 ± .6	41.0 ± .3	45.5 ± .5	44.3 ± .9
$[K]_i$, mEq/l	115.5 ± 5.2	111.6 ± 5.8	135.3 ± 4.8	132.9 ± 5.4
No. of animals	8	8	8	8

± = standard error. Δ = change observed in both experiments and in both tissues.
* $p < .02$.
† $p < .05$.

amount of intracellular sodium (total Na minus extracellular Na) in skeletal muscle and in aorta in the groups receiving PLV-2 compared to their respective controls. Third, the movement of sodium was relatively greater than that of water since both in skeletal muscle and in aorta the calculated ratio of cell sodium/cell water, expressed conventionally as intracellular $[Na]_i$mEq/l, increased. In the case of the aorta this increase was significant in both experiments. There was no measurable change in either the amount or relative distribution of K.

Acute effects of intravenous PLV-2 on blood Na^+ and K^+. Doses of 3, 5, 10, and 20 mU of PLV-2 in 0.2 ml of plasma were injected into the left femoral vein in sequence in each of 6 rats. Each sequence was preceded by injection of plasma as control. Each drug injection was allowed to run its course of about 15 minutes before the next was started. The results of a typical series are shown in Fig. 1.

Consistent with the chemical analytic findings, the injection of PLV-2 produces a fall in blood Na^+ and a corresponding rise in

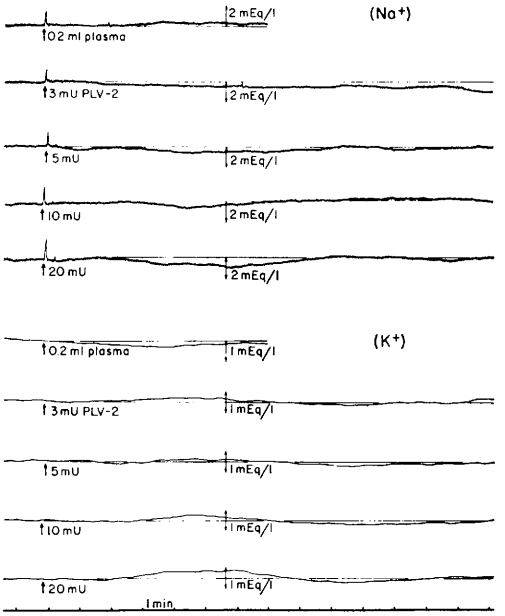

FIG. 1. Responses of arterial blood Na^+ and K^+ activity to a series of intravenous doses of PLV-2 each in 0.2 ml of plasma. Spontaneous baseline variation did not exceed 0.3 mEq/l for Na^+ activity and 0.15 mEq/l for K^+ activity during this run.

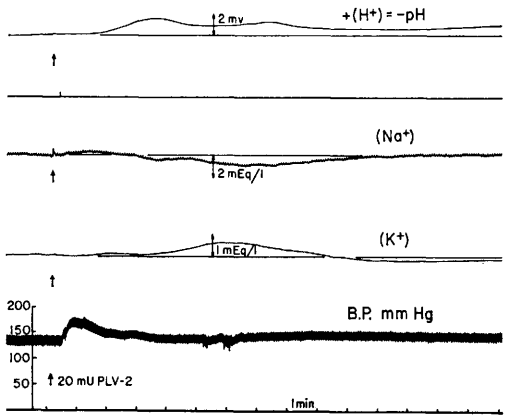

FIG. 2. Responses of arterial blood H^+, Na^+ and K^+ activity and blood pressure following administration of 20 mU of PLV-2 intravenously.

blood K^+. These effects are discernible with 3 mU, a minimal pressor dose. The primary response lasts about 10 minutes and is then followed by a secondary oscillation. The peak of the primary response occurs 5 to 7 minutes after the intravenous injection. With the highest dose of PLV-2 there is a suggestion that Na^+ activity rises first for a short period before entering its steep fall.

Effects of PLV-2 and of Syntocinon on blood Na^+, K^+, and H^+. The effects of 20 mU of PLV-2 were compared with those of 20 mU of Syntocinon in 6 experiments in which complete records for blood Na^+, K^+, and H^+ and blood pressure were obtained in the intact rat. In each case, 0.1 ml of fresh plasma was first injected intravenously as control. This was followed 15 minutes later by Syntocinon similarly administered and observed for 30 minutes and then by PLV-2 observed for a similar 30-minute period.

A typical result for PLV-2 is shown in Fig. 2. The primary fall in Na^+ and rise in K^+ followed by a gradually tapering damped oscillation is as previously observed. A large and sustained increase in H^+ (fall in pH) begins synchronously with the first shift of Na^+, reaches a maximum about 3 minutes after injection, then progressively returns to normal. Addition of H^+ to the blood is inverse in pattern (but not in degree) to the subtraction of Na^+. The curves for blood Na^+, K^+, and H^+ with Syntocinon, which at this dose is minimally pressor, were similar in shape to those observed with PLV-2 but of threshold degree.

Discussion. Systemic administration of a synthetic vasopressin, PLV-2, produces a dynamic exchange of sodium, potassium, and hydrogen in the rat. The change consists essentially of a movement of sodium and water out of blood and extracellular fluid and into cells in the bulk tissue of the body as represented by skeletal muscle and aorta samples. Sodium moves in relative excess of water so that a fall in blood Na^+ can be consistently monitored. There is some indication, however, that water moves first, before sodium, for in the first few minutes following injection of the hormone a small but consistent rise in blood Na^+ is observed. No real movement of potassium between cells and environment can be measured, but since water does shift, a rise in blood K^+ is a significant feature of the response. Except for the fact that H^+ activity has not previously been measured, these results are entirely in agreement with those described by ourselves and by others using crude vasopressor extracts(1). They have here been obtained at low dose levels with pure hormone.

The acute effect of PLV-2 on water distribution is consistent with the observation that chronic administration of vasopressin also increases cell water at the expense of the extracellular environment and with the reverse distribution observed in diabetes insipidus(7). The fact that a pressor dose of PLV-2, or for that matter vasopressin, moves Na into cells is not, however, consistent with the fact that chronic administration of vasopressin produces a loss of cell Na or that a gain of Na is a feature of diabetes insipidus(8). This inconsistency would be resolved if the primary effect of the acute administration of vasopressin *in vivo* were on the distribution of water as it seems to be *in vitro*(9). In this case "solvent drag" might pull Na temporarily into cells to be followed in the long term by gradual elimination of the excess Na.

We were led to measure H^+ activity in these experiments because of the suggestion in chemical work with small arteries that ion exchange mechanisms might be involved(4). Dubuisson(10) has observed an increase in

extracellular H^+ during contraction of frog stomach smooth muscle and more recently Kulbertus(11) has observed this in carotid artery strips. Both of these authors have attempted to relate this change to metabolic events. The pattern of H^+ increase which we have monitored in association with vasoconstriction seems to be related to Na^+ exchange, however, and this suggests to us that ion exchange processes rather than metabolic events may be responsible.

Summary. PLV-2, a synthetic vasopressin, administered subcutaneously in the nephrectomized rat, produces a shift of water and, in greater degree, of Na out of the extracellular space and into cells as measured chemically in aorta and skeletal muscle. No shift of K can be measured. When arterial blood in the intact rat is monitored with glass electrodes following intravenous injection of PLV-2, the shift of Na in excess of water into cells is reflected by a rise in blood K^+. Since these changes are associated with an increase in blood H^+ it is suggested that ion exchange processes may be involved.

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1. Friedman, S. M., Friedman, C. L., Handbook of Physiology, Sect. II, Circulation, 1963, v2, 1135.
2. Jamieson, J. D., Friedman, S. M., Circulation Res., 1961, v9, 996.
3. Daniel, E. E., Dodd, A., Hunt, J., Arch. intern. pharmacodynamie, 1959, v119, 43.
4. Friedman, S. M., Nakashima, M., Friedman, C. L., Proc. Soc. Exp. Biol. and Med., 1964, v117, 480.
5. Sréter, F. A., Woo, G., Am. J. Physiol., 1963, v205, 1290.
6. Bowers, F. K., Friedman, S. M., Biomed. Sci. Instrumentation, 1964, v2, 173.
7. Friedman, S. M., Sréter, F. A., Nakashima, M., Friedman, C. L., Am. J. Physiol., 1962, v203, 709.
8. Friedman, S. M., Friedman, C. L., Canad. J. Physiol. Pharmacol., 1965, v43, 699.
9. Leaf, A., Hays, R. M., J. Gen. Physiol., 1962, v45, 921.
10. Dubuisson, M., J. Physiol., 1939, v94, 461.
11. Kulbertus, H., Angiologica, 1964, v1, 275.

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Synthesis of Deoxyribonucleic Acid in the Liver of Hypophysectomized Rats After Partial Hepatectomy.* (30499)

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The role of the pituitary in liver regeneration following partial hepatectomy is not clearly understood. Cater *et al*(1) were able to demonstrate a prolongation of DNA synthesis and mitotic activity in partially hepatectomized rats following somatotropin injections. The peak of mitotic activity shifts in time following hypophysectomy (Hemingway and Cater(2), Weinbren(3)). Mitotic activity and growth need not follow a directly proportional course in an organ having cells of different stages of ploidy; however, it should be possible to more accurately define the effect of hypophysectomy on liver regeneration by determining the rate of synthesis or turnover of DNA.

Method. Sprague-Dawley rats weighing 180 g were hypophysectomized by the technique described by Reiss *et al*(4). Thirty days later, ⅔ of the liver was removed from the animals. Non-hypophysectomized control rats of the same age were also partially hepatectomized at the same time. At varying intervals following hepatectomy, the rats received intraperitoneally 0.8 μc thymidine-6-T(n) (Radiochemical Centre of Amersham, specific activity 1.9 C/mM) per g body weight. The animals were sacrificed 60 minutes later and the liver was removed and

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