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Antidiuretic Activity of Terlipressin (Triglycyl–Lysine Vasopressin) – Role of Pressure Natriuresis

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Summary

Terlipressin (triglycyl-lysine vasopressin TP), a "hormonogen" analogue, was introduced in gastroenterology for its low and protracted vasopressor action, reducing bleeding from gastrointestinal tract. Its antidiuretic activity, estimated originally in ethanol-anaesthetized rats (Sawyer's method) was claimed to be equally low and protracted. We performed several series of antidiuretic tests on conscious rats (Burn's method) with the following results. TP in low doses of 0.05 – 1.0 μg/kg exhibited typical dose-dependent antidiuretic effect. In the dose of 0.2 μg/kg, the dynamics of urine and sodium excretion did not differ from that after equivalent dose of lysine vasopressin and equipotent dose of DDAVP. The antidiuretic potency of TP (estimated by parallel line assay) was 175.0 U/mg. TP in doses of 5.0 and 20.0 μg/kg exhibited limited diuresis and marked natriuresis. High osmolality and sodium content were present in all portions of excreted urine. The discrepancy between previous and our results concerning antidiuretic activity of TP and the role of pressure natriuresis for overall renal action of TP are discussed.

Key words

Terlipressin – Antidiuretic activity – Pressure natriuresis

Introduction

Terlipressin (triglycyl-lysine-vasopressin, (Remestyp Spofa) was the first synthetic analogue of neurohypophyseal hormones of Czechoslovak origin, successfully introduced in clinical practice – gastroenterology. The other two are Methyloxytocin Spofa in obstetrics and desmopressin (DDAVP, Adiuretin Spofa), superior in the treatment of cranial diabetes insipidus and a drug of choice in haematological and neurological disorders.

Terlipressin (TP) was synthesized in the sixties (Kasafírek *et al.* 1965), when the structure – function relationship of analogues with extended N-alpha-side chain was studied. These analogues were called "hormonogens" according to their ability to release slowly the parent hormone by enzymatic action – as was proved in *in vitro* systems (Beránková-Ksandrová *et al.* 1964) – and thus to exert protracted biological effects. Among them TP exerted the most favourable properties, especially low and protracted vasopressin activities, both pressor and antidiuretic. They were estimated by Kynčl *et al.* (1966, 1974), the pressor activity on pithed rat according to Dekanski (1952), the antidiuretic activity on ethanol-anaesthetized rat (Sawyer 1958, Pliška and Rychlík 1967). Both were calculated to be 2.1 and 2.7 U/mg, respectively, of two orders of magnitude lower than those of lysine-vasopressin (250-270 IU/mg, 3rd International Standard of Oxytocic, Vasopressor and Antidiuretic Substances) with the index of persistence (Řežábek and Kynčl 1966) about 5. The difference between pressor responses to lysine-vasopressin (LVP) and TP in pithed rat is demonstrated in Fig. 1. Such a mild and prolonged vasopressor action stimulated many experimental haemodynamic studies, confirming the advantages of TP over the brisk and short effect of LVP (for review see Jošt 1988a,b). For its therapeutic use, the most relevant was prolonged vasoconstriction in the upper splanchnic region, reducing bleeding from experimental gastric ulcers and esophageal varices, besides a low toxicity and cardiotoxicity. After a lot of good results in clinical trials (Jošt 1988b), TP became a constant part in complex therapy of haemorrhagic disorders in gastroenterology. In this indication, it

replaced LVP which sometimes exerted unfavourable side effects.

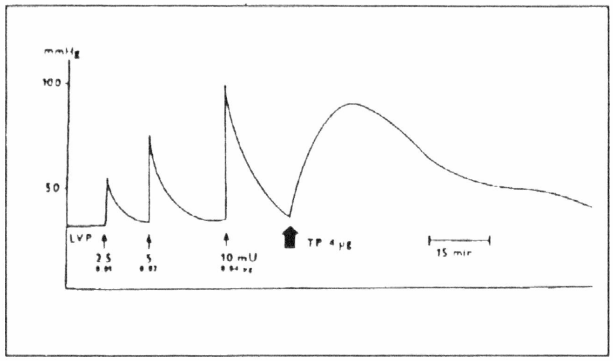

Fig. 1
The record of pressure responses to LVP and TP in pithed rat (male Wistar-Hannover, 450 g). The doses mentioned were applied per rat.

As already mentioned, the antidiuretic activity of TP was claimed to be equally low and protracted as the pressor one (Kynčl *et al.* 1966, 1974). This was considered to be another advantage of this compound and the value of 2–3 U/mg was cited in all papers concerning TP. Nevertheless, Barth *et al.* (1975) observed quite potent ability of TP to stimulate renal adenylate cyclase and concluded that it should have considerable antidiuretic activity of its own. Yet the relations between TP and antidiuresis were thoroughly studied from another point of view. Several authors described the appearance of antidiuretic "material" or "activity" in urine or plasma of cats, rats, healthy volunteers and patients with cirrhosis of the liver after the administration of TP (Kynčl and Rudinger 1970, Pliška *et al.* 1980, Forsling *et al.* 1980). This material resembled LVP by bioassay – at those times it was impossible to differentiate between TP and LVP by RIA – and these experiments served as a support of "hormonogenic" nature of TP. We therefore wondered how this interesting action would appear in the antidiuretic test in conscious rats. We carried out several series of experiments with different doses of TP. As a standard preparation we used LVP and we also applied DDAVP as another peptide with protracted antidiuretic action. Because the first results were rather unexpected, we tried to elucidate the renal action of TP more precisely.

Material and Methods

The experiments were performed in male albino rats of the Wistar–Hannover strain with average body weight of about 200 g, accustomed to experimental procedures. Antidiuretic tests were done by a modification of Burn's method (Burn *et al.* 1950) whose principle is the delay of water diuresis induced by a single water load, due to simultaneous administration of vasopressin. The magnitude of antidiuresis was expressed as "half-time" ($t_{1/2}$), that means the time in minutes, when half of the water load was excreted. After overnight fasting with water ad libitum, the rats were given tap water by a metal stomach tube in a volume equal to 4 % of body weight. The peptides were administered by subcutaneous injection, the definite dose in a volume of 2 ml/kg. The rats were subsequently placed in individual metabolism cages for five hours. The urine was collected in calibrated tubes, its volume was recorded every 15 minutes and expressed as % of a given water load. Further analyses of the urine were performed according to the experimental design.

Experiment I. TP was given in doses of 0.05; 0.2; 1.0; 5.0; 10.0 and 20.0 µg/kg. LVP as a standard, was administered in the dose of 0.2 µg/kg (equivalent to 50.0 mU/kg) and DDAVP in an equipotent dose of 200 pg/kg (2×10^{-7} mg). In this experiment only values of $t_{1/2}$ were calculated, if possible.

Experiment II. On the basis of previous experiment, TP in a dose of 0.2 µg/kg was compared with LVP and DDAVP (0.2 µg/kg and 200 pg/kg, respectively) and also two high doses of TP, 5.0 and 20.0 µg/kg were applied. The experiment was performed twice in this arrangement. For the first time, urinary sodium (U_{Na}), potassium (U_K) and osmolality (U_{osm}) were measured in the first portion of excreted urine, and on the top of water diuresis or in the second portion of urine. For the second time, the total amount of sodium ($U_{Na}V$), potassium (U_KV) and endogenous creatinine ($U_{Cr}V$) excreted during the five hours' experiment were determined.

Experiment III. TP and LVP were applied in the dose of 0.2 µg/kg and five times lower dose of 0.04 µg/kg; each dose, including the controls, to 8 animals. The relative antidiuretic potency of TP was calculated from the values of $t_{1/2}$ by means of parallel line assay with 95 % fiducial limits.

TP was used in the dispensing form Remestyp Spofa inj., batch. 030388, LVP in the form of laboratory standard preparation, amp. a 1.0 U/ml, batch. 130489, DDAVP in the form of laboratory standard for Adiuretin Spofa, amp. a 30.0 µg of substance. Urine osmolality was measured by cryoscopy (digital osmometer Knauer), urinary sodium and potassium by

flame photometry (FLM 3 Radiometer Copenhagen), urine endogenous creatinine by photometry (Specol Zeiss, Jena). Statistical evaluation of differences between means was performed by the t test, $p < 0.05$.

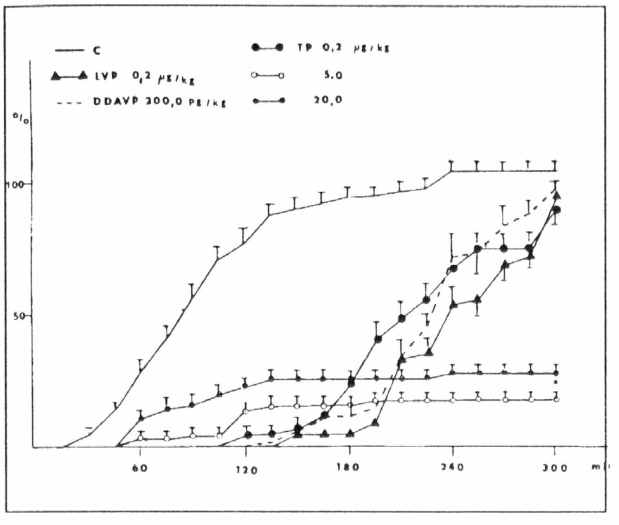

Fig. 2
Cumulative curves of diuresis after TP, LVP and DDAVP during five hours of the experiment. The points represent means ± S.E.M. in 15 min intervals. % – the amount of excreted urine expressed as a percentage of a given water load. * – significant difference between the total urine volumes.

Results

Experiment I. TP in doses of 0.05, 0.2 and 1.0 μg/kg exhibited the typical dose-dependent antidiuretic response. After the dose of 0.05 μg/kg, the $t_{1/2}$ was about 150 min, whereas after the dose of 1.0 μg/kg almost complete antidiuresis was observed during the whole experiment. After the medium dose of 0.2 μg/kg, the duration of antidiuresis and the dynamics of subsequent diuresis resembled those after LVP and DDAVP with no statistical difference between their values of $t_{1/2}$, as will be demonstrated in Fig. 2 and Tab. 2 and 3. After the doses of TP 5.0 μg/kg and higher, a qualitatively different response appeared – the initial diuresis of a "pulsatile" character and limited duration (Fig. 2). Each animal excreted only two or three portions of urine within the first three hours of the experiment, the urine volume being always greater after the higher dose. Only exceptionally, more than 50 % of the water load was excreted even after the highest dose (20.0 μg/kg). The values of $t_{1/2}$ could not be determined anyway.

Table 1
The values of urine osmolality, sodium and potassium in the first portions of excreted urine

	Dose/kg	ml	U_{osm}	U_{Na}	U_K
C	---	1.2 ± 0.2	210 ± 24	16 ± 11	32 ± 4
LVP	0.2 μg	1.9 ± 0.2	851 ± 35*	22 ± 10	39 ± 6
DDAVP	200 pg	1.5 ± 0.2	922 ± 72*	19 ± 5	36 ± 6
TP	0.2 μg	1.8 ± 0.2	920 ± 98*	31 ± 10	47 ± 6
	5.0 μg	1.3 ± 0.1	974 ± 60*	170 ± 28*,a,c	77 ± 9
	20.0 μg	1.2 ± 0.2	753 ± 63*,b	205 ± 15*,a,c	51 ± 6

Means ± S.E.M; ml = mean volume of the first portion
Significantly different: * - from the controle, a - from TP 0.2 μg/kg, b - from TP 5.0 μg/kg and c - from LVP

Table 2

A – The value of t½, urine osmolality, urine sodium and potassium at the peak of water diuresis. B – The same values in the second portion of the excreted urine

		Dose/kg	ml	t½	U_{osm} mosm/kg	U_{Na} mmol/l	U_K	V%
A	C	---	0.5	84 ± 3	99 ± 8	2 ± 1	3 ± 1	103 ± 6
	LVP	0.2 μg	0.5	231 ± 4*	163 ± 17*	3 ± 1	4 ± 1	92 ± 4
	DDAVP	200 pg	0.5	231 ± 7*	163 ± 16*	6 ± 1	4 ± 1	98 ± 4
	TP	0.2 μg	0.5	214 ± 9*	174 ± 20*	6 ± 1	5 ± 4	88 ± 4
B	TP	5.0 μg	1.9 ± 0.2	---	969 ± 154	230 ± 46	100 ± 17	16 ± 2
		20.0 μg	2.4 ± 0.3	---	844 ± 49	259 ± 15	61 ± 6	27 ± 3

Means ± S.E.M. * - significant difference from the controls.
At the peak of water diuresis, 0.5 ml were withdrawn for determination.
The values from part B are not statistically comparable with those from part A.
The total amount of urine at the end of a five hours' experiment is expressed as % of the given water load = V%.

Table 3

A - The values of t½ V%, sodium and potassium excretion and their ratio in the urine and exogenous creatinine excretion during the five hours' experiment and of potasasium at the peak of water diuresis. B - the same values in the course of diuresis.

		Dose/kg	t½min	$U_{Na}V$ μmol/5h	U_KV	Na/K	UcrV μmol/5h	V%
A	C	---	76 ± 4	81 ± 16	84 ± 20	1.00 ± 0.1	11 ± 0.1	103 ± 5
	LVP	0.2 μg	224 ± 7*	112 ± 22	99 ± 12	1.12 ± 0.2	12.8 ± 1.7	89 ± 7
	DDAVP	200 pg	228 ± 6*	65 ± 20	104 ± 14	0.57 ± 0.1c	11.9 ± 0.7	74 ± 3
	TP	0.2 μg	201 ± 9*	65 ± 10	119 ± 9	0.58 ± 0.1c	15.5 ± 0.6*	97 ± 3
			Duration of diuresis (in min)	μmol/time			μmol/time	
B	TP	5.0 μg	189 ± 32	490 ± 42	198 ± 23	2.47 ± 0.2	8.4 ± 0.1	33 ± 3
		20.0μg	161 ± 21	926 ± 97b	251 ± 24b	3.76 ± 0.9	7.3 ± 1.0	60 ± 1

Means ± S.E.M. * - significant difference from the controls, c - significant difference from LVP.
Part A and B are not statistically comparable.

Experiment II. Cumulative curves of diuresis are depicted in Fig. 2, the amount of excreted urine is expressed in % of a given water load. The difference between the "antidiuretic" dose of TP 0.2 μg/kg and those eliciting the limited diuretic response, is clearly visible. On the contrary, there was no substantial difference between the low dose of TP, standard and weight-equivalent dose of LVP and equipotent dose of DDAVP. Their values of $t_{1/2}$ and the total amount of urine excreted during 5 hours of the experiment are given in Tab. 2. After two high doses of TP, 5.0 and 20.0 μg/kg, the diuresis started much earlier but its volume was limited – in this case no animal excreted 50 % of the water load – Tab. 2. The initial urinary osmolality (U_{osm}) and sodium and potassium concentration (U_{Na}, U_K) in the first portion of urine, excreted after an appropriate delay or antidiuresis, are demonstrated in Tab. 1. The initial urinary osmolality was equally elevated after all doses of peptides, but the amount of initial urine sodium was markedly lower in the typical "antidiuretic" responses.

The same values are demonstrated in Tab. 2. On the top of water diuresis (part A) the marked dilution of urine occurred with no difference in U_{Na}, and with a slight but significant difference in U_{osm} between control and LVP, DDAVP and TP 0.2 μg/kg. This was not the case after TP 5.0 and 20.0 μg/kg (Tab. 2, part B) where urine sodium and osmolality, and to some extent also urinary potassium, remained elevated as in the beginning. In this arrangement the experiment was repeated for the second time, in order to assess the total excretion of sodium and potassium ($U_{Na}V$, U_KV) during five hours after administration of peptides. Endogenous creatinine excretion ($U_{Cr}V$) was determined as a rough estimation of changes in glomerular filtration. The results are given in Tab. 3. The dynamics of urine excretion was the same as in the former experiment. The total sodium, potassium and endogenous creatinine excretion after LVP, DDAVP and TP 0.2 μg/kg did not differ substantially from those of the controls (Tab. 3A). As expected, the two high doses of TP, 5.0 and 20.0 lg/kg elicited marked and dose-dependent sodium diuresis, limited to 2–3 hours of the experiment (Tab. 3B) Because these doses already elevate the systemic blood pressure (Trčka 1966) and probably also renal perfusion pressure, it was obvious that pressure natriuresis masked the antidiuretic effect of TP. In this experiment, for the first time the animals excreted more than 50 % of water load after the highest dose of TP.

Experiment III. According to previous results, the antidiuretic activity of TP had to be close to 200.0 U/mg. We made therefore an attempt to determine it more precisely by comparing two equivalent doses of TP and LVP. The values of $t_{1/2}$ after the doses of 0.04 and 0.2 μg/kg of both peptides are shown in Tab. 4.

Table 4
The values of t½ after two equivalent doses of TP and LVP

t½	Dose/kg
C	---
68.6 ± 44	
LVP	0.04 μg
139.4 ± 10.2	
	0.2 μg
186.1 ± 10.7	
TP	0.04 μg
111.0 ± 7.0	
	0.2 μg
187.7 ± 9.0	

Means ± S.E.M. All values are significantly different from the controls.
* - significant difference from the corresponding dose of LVP.

The values after the dose of 0.2 μg/kg were almost identical, whereas those after the dose of 0.04 μg/kg differed significantly, indicating nonparallelism of dose – response curves. (Indeed there was the statistically significant nonparallelism in the experiment performed on another group of rats – data not shown.) The estimation of relative antidiuretic potency of TP could be therefore determined only approximately as 175.0 U/mg with fiducial limits 100.0 to 268.0.

Discussion

The results of our experiments pointed out two interesting renal effects of terlipressin. First, the antidiuretic activity of TP in low doses was almost as high as that of LVP. Second, TP in high doses elicited pressor

natriuresis which masks its antidiuretic action. Both deserve some comment.

The discrepancy between the previous estimation of antidiuretic activity of TP – about 3.0 U/mg (Kynčl *et al.* 1966, 1974) and our results – about 200.0 U/mg can be explained by differences in methods and dosage as well as by differences in the interpretation. The method using ethanol-anaesthetized, continually hydrated rats with cannulated urinary bladder (Sawyer 1958) is elegant, sensitive and suitable for low threshold doses. At the time of its introduction it was an evident progress compared to Burn's method (Burn *et al.* 1950). This latter one is more simple, is performed on conscious hydrated rats, requires higher doses with distinct activity equal to 10.0–50.0 mU/kg and does not recognize at all the prolonged activity of tested compounds. (For instance in Fig. 2, the cumulative curve of diuresis after DDAVP, whose prolonged action is well documented, was the same as that after shortacting LVP.) On the other hand, the test is not so stressful for animals and can be performed on several groups of rats simultaneously.

As demonstrated in Experiment III., the dose-response curves for TP and LVP are not parallel, the lower dose of TP being less active than an equivalent dose of LVP. It is therefore possible that low doses of TP, applied by Kynčl *et al.* (1966) to anaesthetized rats were less potent than those in our experiments. We suppose, however, that the main difference is caused by a different interpretation of the results. TP and similar analogues were the first ones with protracted action. Their potency was expressed by two values: one denoting the reduction of drop count (and/or the elevation of urine conductance), the other – index of persistence – the duration of the effect. Later on, when more potent analogues with prolonged action were synthesized (especially DDAVP) it became more convenient to express their potency only by one value, comprising both the effect and its duration (Vávra *et al.* 1968) and to return to Burn's method for testing higher doses (Vávra *et al.* 1974).

Pressure natriuresis is a well-known phenomenon occurring in situations when renal perfusion pressure is elevated. It is mainly caused by inhibition of sodium reabsorption in deep nephrons (Haas *et al.* 1986) and participates in "escape" from the water-retaining effect of vasopressin (Hall *et al.* 1986) and sodium-retaining effect of mineralocorticoids (Knox *et al.* 1980). The pressure natriuresis after high doses of TP should be ascribed to its mild and prolonged vasopressor action. It was impossible to elicit it by a single high dose of LVP (our unpublished results), but prolonged infusion of low doses of AVP did result in pressure natriuresis (Hall *et al.* 1986). This effect of TP might be beneficial for patients with bleeding episodes from the gastrointestinal tract, as it was for laboratory animals in oligaemic shock (Trčka 1966). At least, it helps to support renal circulation in critical situations.

Our results and conclusions are only of theoretical value after all. TP was introduced in clinical practice (instead of LVP) more than a decade ago and became a constant part of the complex therapy of bleeding gastroduodenal ulcers and esophageal varices, without any serious adverse effects. Because it is administered in emergency situations only, there is no danger of water retention or sodium loss.

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