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THE EFFECT OF SOME 2-O-ALKYLTYROSINE ANALOGUES OF OXYTOCIN AND LYSINE VASOPRESSIN ON THE BLOOD PRESSURE OF THE RAT, RABBIT, AND CAT

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2-O-Methyltyrosine-oxytocin, formally related to oxytocin by methylation of the hydroxyl group in the tyrosine side-chain, inhibits the pressor effect of vasopressin in the rat (Law & du Vigneaud, 1960) and, under some conditions, the uterotonic action of oxytocin *in vitro* (Beránková, Rychlík, Jošt, Rudinger & Šorm, 1961 ; Rudinger & Krejčí, 1962 ; Bisset, 1962 ; Krejčí, Poláček & Rudinger, 1967).

The corresponding derivative of lysine vasopressin, 2-O-methyltyrosine-8-lysine-vasopressin, has been prepared by Siedel, Sturm & Geiger (1963) and also by Zaoral, Kasafírek, Rudinger & Šorm (1965) (see Šorm, 1961). Vogel & Hergott (1963) reported that this compound, while possessing some pressor activity, inhibited the pressor action of vasopressin in the rat. In decerebrate cats and rabbits it showed a greater pressor effect but no inhibition was recorded. The compound inhibited the action of oxytocin on the rat uterus *in vitro* but retained a vasopressin-like antidiuretic effect. Essentially similar results were reported by Zaoral & Šorm (1964) who also gave preliminary results for the properties of the higher homologue, 2-O-ethyltyrosine-8-lysine vasopressin. A more complex O-alkylated derivative, N^{α}-glycyl-2-O-methyltyrosine-8-lysine-vasopressin (Zaoral, Pliška, Řežábek & Šorm, 1963 ; Zaoral & Šorm, 1965) was found to have protracted antidiuretic activity in the hydrated rat and a vasodepressor effect, with inhibition of the pressor response to vasopressin, in the rat preparation of Dekanski (1952).

This paper reports the results obtained in a more detailed study of the effects of these O-alkylated analogues on the blood pressure of the rat, cat, and rabbit under various conditions.

METHODS

The 2-O-methyltyrosine-oxytocin (methyloxytocin), 2-O-methyl- and 2-O-ethyltyrosine-8-lysine-vasopressins (methylvasopressin and ethylvasopressin), and N^{α}-glycyl-2-O-methyltyrosine-8-lysine-vasopressin (glycyl-methylvasopressin) were synthetic materials purified by countercurrent distribution or ion exchange chromatography (Jošt, Rudinger & Šorm, 1963 ; Zaoral *et al.*, 1963 ; Zaoral & Šorm, 1965). The Czechoslovak National Standard of Posterior Pituitary Extract, standardized against the Third International Standard of Oxytocic, Vasopressor, and Antidiuretic Substances

(Bangham & Musset, 1958), served as the standard except in a few experiments requiring higher doses in which purified synthetic lysine vasopressin, also assayed against the Third International Standard, was used.

The rats were males of the Wistar strain weighing 180–320 g. They were prepared by urethane anaesthesia, after dibenamine (Dekanski, 1952) or without premedication, or pithed under ether anaesthesia. The cats were mostly males weighing 2–4 kg, anaesthetized with chloralose (0.04 g/kg) and phenobarbitone (0.06 g/kg) with or without dibenamine treatment, or decerebrated with trichloroacetic acid (Vogel & Hergott, 1963). The rabbits (males weighing 1.8–3.5 kg) were anaesthetized with urethane (1.25 g/kg intraperitoneally) and premedicated with dibenamine or decerebrated with trichloroacetic acid. All animals were heparinized.

The blood pressure was recorded from the carotid artery with a mercury manometer. The peptides were injected into the femoral vein in a constant volume of saline (0.2 ml. in rats, 0.5 ml. in cats and rabbits). In experiments with dibenamine-treated or pithed rats the doses of standard were 2.5 m-u (S_1) and 5 m-u (S_2) per animal. The doses of methylvasopressin, which itself has pressor action, were chosen so that they did not exceed the pressor effects of the standard; the doses of the other analogues, which have slight or no pressor action, were selected so as to give a statistically significant inhibition of the response to the standard by 30–50%. The responses to two doses of the standard were compared before and after administration of the analogue or isotonic saline in various sequences. In experiments with cats and rabbits, the standard was injected in doses of 20–100 m-u/animal; the doses of the analogues were chosen according to the criteria given above.

RESULTS

The pressor potencies of the various analogues and their inhibitor properties are summarized in Table 1; the effects on the pressor response to vasopressin of dibenamine-treated and pithed rats are shown in more detail in Table 2. Whenever inhibition was observed it was found to be surmountable in the sense that the original response could be matched by increasing the dose of vasopressin (Fig. 1). The duration of the inhibition varied with the dose; after doses of the analogues causing inhibition of the response to vasopressin by 10–20%, recovery of the initial sensitivity was generally complete and fairly rapid whereas after 60–100% inhibition recovery was generally incomplete during the experimental period (2–3 hr). Special features of the responses to the individual peptides are given below.

TABLE 1

EFFECTS OF 2-O-ALKYLTYROSINE ANALOGUES OF LYSINE VASOPRESSIN AND OXYTOCIN ON THE BLOOD PRESSURE OF THE RAT, CAT, AND RABBIT

The approximate (estimated) potencies are given in i.u./mg; n is the number of experiments, 0 denotes no pressor response, + inhibition, ± variable inhibition, − no inhibition up to the dose levels given in the text or footnote. Results for cats and rabbits are summarized regardless of preparation (anaesthesia with or without dibenamine or decerebration), since no distinct differences in response were observed between the different preparations

	Methyl vasopressin			Ethyl-vasopressin			Methyl-oxytocin			Glycyl-methyl-vasopressin		
	n	potency	inh.	n	potency	inh.	n	potency	inh.	n	potency	inh.
Rat (urethane and dibenamine)	21	2·0	+	24	0·1*	+	40	0	+	27	0	+
Rat (pithed)	49	1·5	±	32	0·1–0·3*	+	19	0	+	28	†	±
Cat	13	30–50	−‡	16	5–10	+	11	0	+	7	0	−
Rabbit	16	50–100	−‡	15	5–10	±	16	v. low	−	5	0	−

* Estimated in low response range. † Protracted; evaluation from the maximum intensity of the pressor response is inadequate. ‡ Up to 10 μg.

TABLE 2

INHIBITOR PROPERTIES OF 2-O-ALKYLTYROSINE ANALOGUES OF LYSINE VASOPRESSIN AND OXYTOCIN IN DIBENAMINE-TREATED AND PITHED RATS

Responses to standard after analogue (dose given per animal) and after the same volume of saline as percentages of the original response to standard; mean and 95% confidence limits for groups of 8 animals. *P* is the probability that the results for analogue and saline under any one set of conditions are significantly different.

	Methyl-vasopressin 1·6 μg	Ethyl-vasopressin 2 μg	Methyl-oxytocin 50 μg	Glycyl-methyl vasopressin 50 μg
	Dibenamine-treated rats, urethane narcosis			
Analogue	76·6±9·2*	49·5±11·3†	50·6±9·2	75·8±9·4
Saline	92·5±17·1	93·6±8·6†	89·0±7·3	95·5±15·6
P	<0·1	<0·005	<0·005	<0·025
	Pithed rats			
Analogue	102·6±11·0	79·8±14·2	90·0±10·9	105·0±19·4
Saline	136·4±17·4	140·5±15·7	132·2±19·6	115·3±4·2
P	<0·005	<0·005	<0·005	<0·5‡

* A dose of 8 μg caused inhibition by about 30%; no further increase in inhibition was achieved at higher doses. † 12 animals. ‡ The variability is significantly ($P<0·005$) greater for the analogue.

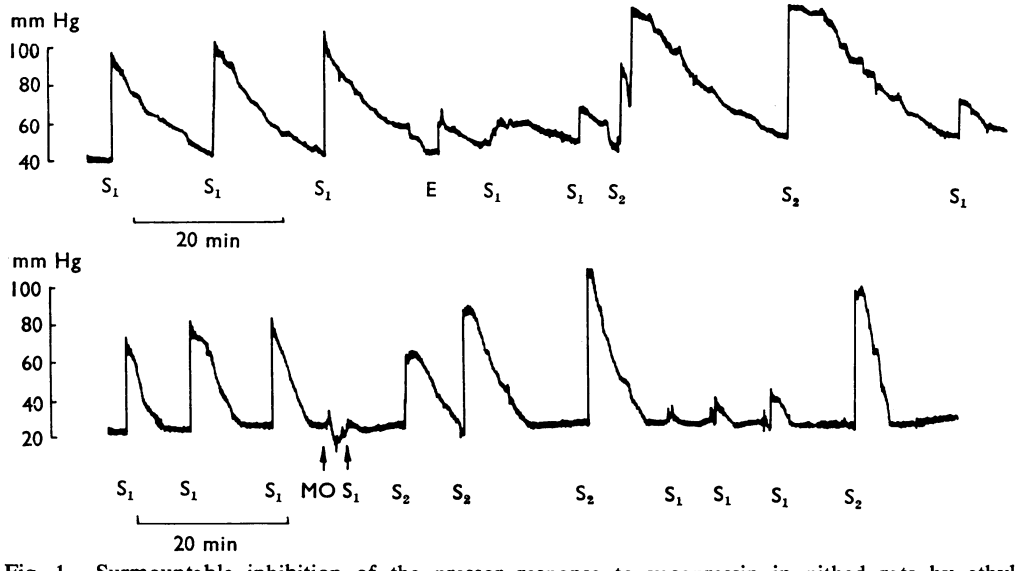

Fig. 1. Surmountable inhibition of the pressor response to vasopressin in pithed rats by ethyl-vasopressin and methyloxytocin. S_1, S_2 lysine vasopressin (5 m-u and 25 m-u, respectively); E ethylvasopressin (25 μg), MO methyloxytocin (100 μg).

Vasopressin

In dibenamine-treated rats the pressor responses to pituitary extract (arginine vasopressin) or to lysine vasopressin decreased somewhat with time but this decrease generally amounted to no more than 10% of the original response over 2 hr. In pithed rats the pressor responses generally increased during 1–1½ hr after pithing and then remained constant for some time or gradually decreased (Fig. 2). This decrease in the second phase was observed in an appreciable proportion of the animals so that no useful purpose

was served by postponing assays until the first phase of increasing sensitivity was completed. In cats and rabbits the sensitivity was relatively low, the dose dependence of the response was less steep than in rats, and tachyphylaxis was more pronounced, so that reliable assays were difficult to perform. The situation was not improved by pharmacological sympathetic blockade or surgical blockade of vasomotor regulation.

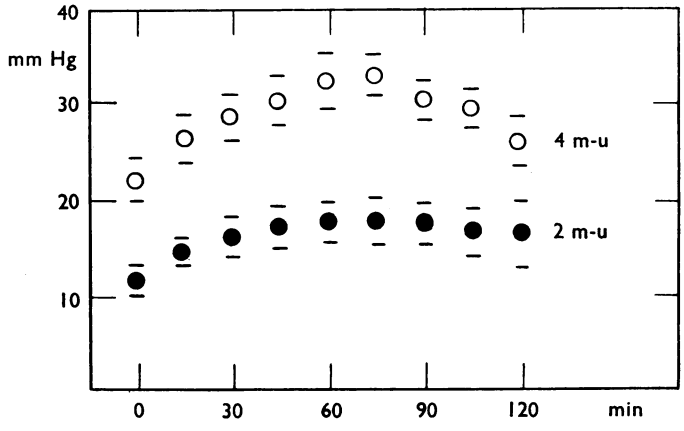

Fig. 2. Time course of the carotid blood pressure response to equal doses of vasopressin in the pithed rat. Standard (pituitary extracted, 2 and 4 m-u/animal) at 15-min intervals. Each set of points is the mean for 10 animals with 95% confidence limits.

Methylvasopressin

In dibenamine-treated rats the loss of sensitivity to both the analogue and the standard was much more marked than with vasopressin alone. Because of this the potency of the analogue (Table 1) could not be determined by the usual assay procedure but had no be estimated with reference to doses of the standard administered before the analogue, by interpolation. In pithed rats the pressor response to vasopressin given after the analogue was unchanged but when vasopressin was given after an injection of saline the response was increased (Table 2). In 21 experiments on rats in urethane narcosis without dibenamine treatment the analogue (8 μg) inhibited the response to subsequent doses of the standard (10–20 m-u) by about 50% ($P<0.01$); no reliable estimate of the pressor potency of the analogue could be made.

Ethylvasopressin

In dibenamine-treated or pithed rats doses of the analogue up to 100 μg caused only a slight pressor reaction, or none at all. Marked inhibition of the response to vasopressin was produced even by 2 μg ethylvasopressin (Fig. 3; Table 2), and 100 μg generally blocked the response to the standard completely. In cats and rabbits no marked difference was observed between the responses of animals in general anaesthesia alone, with dibenamine blockade, or after decerebration. In all instances the response was highly variable, and the potency had to be estimated (Table 1). Inhibition of the response to vasopressin was sometimes observed (in individual cases) after 10 μg or more of the analogue had been given.

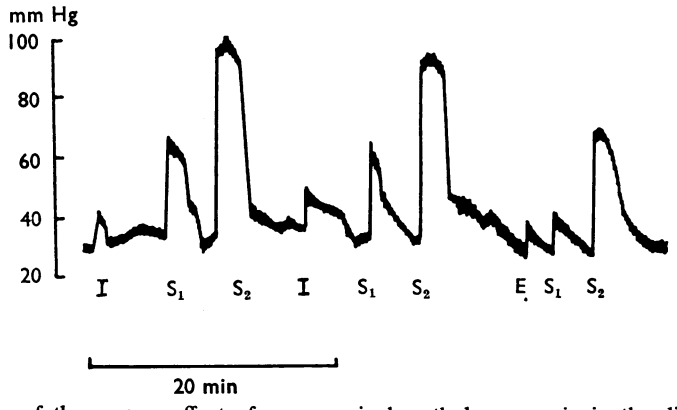

Fig. 3. Inhibition of the pressor effect of vasopressin by ethylvasopressin in the dibenamine-treated rat. S_1, S_2 pituitary extract (2.5 m-u and 5 m-u, respectively); E ethylvasopressin (2 μg); I isotonic saline (0.3 ml.).

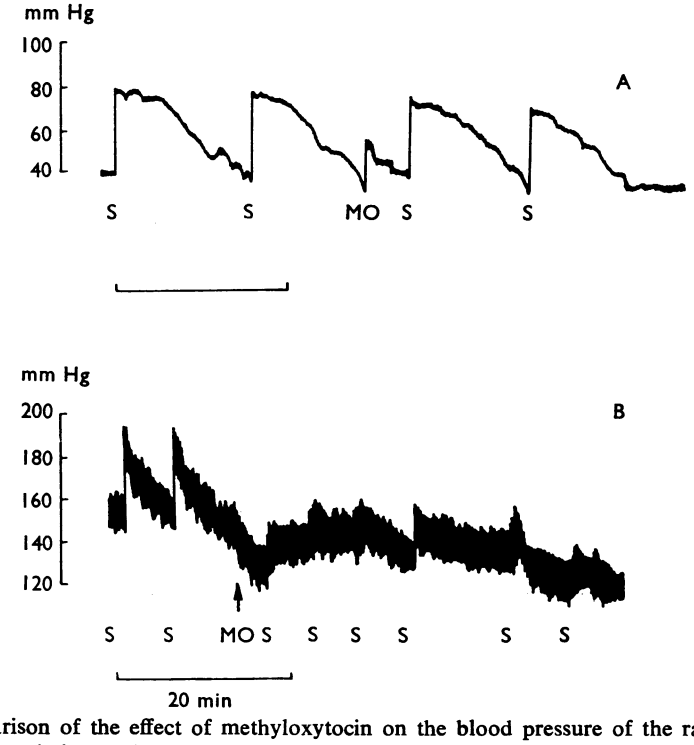

Fig. 4. Comparison of the effect of methyloxytocin on the blood pressure of the rabbit (A) and cat (B). MO methyloxytocin (50 μg); S pituitary extract (50 m-u).

Methyloxytocin

In rats methyloxytocin showed no pronounced pressor activity in doses up to 100 μg; only in isolated instances, all in the dibenamine group, were brief, slight increases in blood pressure observed. Inhibition of the response to vasopressin was less marked in

pithed than in dibenamine-treated rats. The pressor response to oxytocin (10 μg by uterotonic assay) was distinctly inhibited by 20–50 μg methyloxytocin. In cats methyloxytocin showed no pressor effect. In 8 out of 9 anaesthetized and in 1 of 2 decerebrated animals, 50 μg of the analogue markedly inhibited the response to 50 m-u of vasopressin (Fig. 4B). In 13 out of 16 rabbits the analogue had a slight pressor effect, without regular dependence on dose ; no estimate of the potency could be made. Inhibition was indistinct or absent after doses up to 100 μg (Fig. 4A).

Glycyl-methylvasopressin

In agreement with the results of Zaoral *et al.* (1963) we found glycyl-methylvasopressin to cause a brief drop in the blood pressure of dibenamine-treated rats or no change distinguishable from the response to the same volume of saline ; only in 3 experiments out of 14 was there a slight, protracted rise in the blood pressure. The inhibitory effects were relatively weak (Table 2, Fig. 5). In pithed rats, glycyl-methylvasopressin caused a protracted increase in the blood pressure (40–60 min after 50–100 μg) in 12 out of 20 animals ; in the remaining rats the effect of the analogue was similar to that in dibenamine-treated rats. No pressor effect was observed with 4 rats in ethanol narcosis. Of 10 rats in urethane anaesthesia treated with the myorelaxant gallamine (Remyolan® SPOFA) and kept under artificial respiration, only one showed the protracted pressor response to the analogue. No appreciable pressor effects and no inhibitory action was found in cats (doses up to 250 μg) and rabbits (50–100 μg).

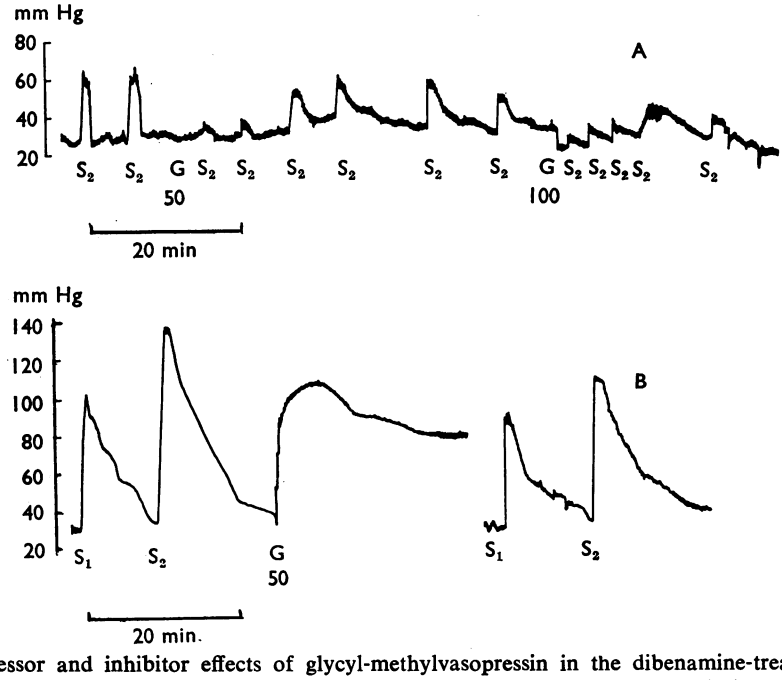

Fig. 5. Pressor and inhibitor effects of glycyl-methylvasopressin in the dibenamine-treated rat (A) and the pithed rat (B). G glycyl-methylvasopressin (doses in μg) ; S_1, S_2 pituitary extract (2.5 m-u and 5 m-u, respectively). The gap in the trace represents 20 min.

DISCUSSION

All the O-alkylated derivatives of vasopressin and oxytocin show common features in their effect on the blood pressure of anaesthetized animals. There is always a marked decrease, or complete disappearance, of the pressor effect in the conventional assay on rats under sympathetic blockade, together with inhibition of the pressor response to subsequent doses of vasopressin. The inhibition by all the analogues, at the doses used, is surmountable by increased doses of vasopressin. The duration of the inhibitory effect is much greater than the persistence of the pressor response to vasopressin, and also to methylvasopressin, ethylvasopressin, and methyloxytocin in those preparations in which each particular analogue has pressor action.

Methylvasopressin shows pressor action and inhibitory properties in association; in ethylvasopressin the pressor activity is largely suppressed, and methyloxytocin generally elicits no pressor response. Glycyl-methylvasopressin is pressor in pithed rats only, whereas in dibenamine-treated rats it has vasodepressor action. Our results with methyloxytocin are in agreement with those recorded by Law & du Vigneaud (1960).

The effect of central denervation in rats produced marked changes in the character of the response only with glycyl-methylvasopressin (see below). The decreased inhibitory action observed with the other analogues is apparent only. If the increasing sensitivity of the preparation is allowed for (Fig. 2 and responses to vasopressin after saline in Table 2), the inhibitory effects are found to resemble those in the dibenamine-treated rats. We have no explanation to offer for this increasing sensitivity to vasopressin after pithing in the rat. No analogous phenomenon was observed after decerebration in cats or rabbits; however, we have found similar changes in the response of pithed rats to adrenaline and noradrenaline (B. Kupková, unpublished results).

The properties of glycyl-methylvasopressin are somewhat complex. Analogues of oxytocin and vasopressin with the peptide chain extended have been shown to act as "hormonogens," releasing the appropriate hormone by enzymic action *in vivo* (Beránková-Ksandrová, Bisset, Jošt, Krejčí, Pliška, Rudinger, Rychlík & Šorm, 1966). A similar situation would be expected with glycyl-methylvasopressin; however, in dibenamine-treated rats this analogue was generally inactive, or produced a brief drop in the blood pressure, and the expected protracted pressor effect only emerged when pithed rats were used. This difference might be explained by the increased sensitivity of pithed rats to vasopressin (and methylvasopressin) resulting from the greater initial vasodilatation. In the less sensitive dibenamine-treated rat, the rate of release of methylvasopressin from the hormonogen might be insufficient to reach the threshold for pressor action, but sufficient to induce tachyphylaxis, and the associated loss of sensitivity to vasopressin. The brief vasodepressor effect seems to be a property of the analogue itself. However, the difference in sensitivity between the pithed and dibenamine-treated rat appears to be an insufficient explanation for so marked a change in response, particularly in view of the fact that in cats and rabbits, where methylvasopresssin has a much higher pressor activity than in rats, there is no pressor response, protracted or otherwise, to the glycyl derivative. Nor does glycyl-methylvasopressin have pressor activity in ethanol-anaesthetized rats, although under similar conditions the analogue has a protracted antidiuretic effect in hydrated animals (Zaoral *et al.*, 1963). Finally, the possibility that the artificial respiration used with pithed rats, and the resultant difference in the acid–base

balance, might be the cause of the difference in response, as in the effect of alkalosis on the vasoconstrictor action of noradrenaline (Bygdeman, 1963), was checked in experiments with urethane-anaesthetized rats treated with a muscle relaxant and kept under artificial respiration. Only in one experiment out of 10, however, did glycyl-methylvasopressin evoke a protracted rise in blood pressure. The conditions determining the response to this analogue thus remain obscure and will require further study.

In general, the pressor properties may be said to decrease from methylvasopressin through ethylvasopressin to methyloxytocin, as regards both potency and the range of preparations which show a pressor response. Among the species examined, the sensitivity of the vascular response (represented by the mean arterial pressure) to the structural changes in this series of analogues is greatest in the rat and least in the rabbit (Table 1). These observations once again emphasize the necessity for using several species in studying the effects of neurohypophysial hormone analogues on the circulation.

SUMMARY

1. The effects of a number of O-alkylated analogues of oxytocin and lysine vasopressin on the blood pressure were studied in anaesthetized rats, cats and rabbits either under pharmacological sympathetic blockade or under central denervation.

2. The pressor activities decreased in the order 2-O-methyltyrosine-8-lysine-vasopressin, 2-O-ethyltyrosine-8-lysine-vasopressin, 2-O-methyltyrosine-oxytocin, and N^{α}-glycyl-2-O-methyltyrosine-8-lysine-vasopressin. The oxytocin analogue was inactive in almost all the preparations. The glycyl derivative, which probably acts by releasing 2-O-methyltyrosine-vasopressin, was inactive in dibenamine-treated rats but evoked a protracted pressor response in pithed rats.

3. All the analogues inhibited the pressor action of vasopressin in rats, irrespective of the pretreatment of the animals.

4. Both the decrease in pressor activity of the analogues (as compared with the parent hormones) and the inhibitory effects were more pronounced in rats than in cats or rabbits.

We are greatly obliged to Dr. M. Zaoral, Mr. E. Kasafírek, Dr. K. Jošt and Professor J. Rudinger for providing the purified lysine vasopressin and the vasopressin and oxytocin analogues used in this work. To Professor J. Rudinger we are indebted for numerous discussions and for the translation of this paper.

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