Effects of Lysine-Vasopressin (LVP) and 1-Deamino-8-D-Arginine-Vasopressin (dDAVP) upon Electrical Potential, Short-Circuit Current and Transepithelial D.C. Resistance of the Frog Skin

P. BAKOŠ, J. PONEC and B. LICHARDUS

Institute of Experimental Endocrinology, Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Czechoslovakia

Abstract. The synthetic analogue of vasopressin, 1-deamino-8-D-arginine-vasopressin (dDAVP), possesses a protracted antidiuretic activity while having practically no pressoric activity as compared to arginine-vasopressin (AVP) or lysine-vasopressin (LVP). The effects of LVP and dDAVP were studied on the frog skin (*Rana temporaria*) sodium transport as reflected by the short-circuit current (SCC) level, on an Ussing apparatus. The application two different equimolar doses of LVP or dDAVP (approx. 9.4×10^{-8} mol . l^{-1} and 18.8×10^{-8} mol . l^{-1}) to the inner surface of the skin resulted in identical maximal increases of sodium transport. However, the maximum transport stimulation after the application of dDAVP was delayed by about 30 min as compared to the stimulation by LVP ($P < 0.01$). In addition, a protracted recovery of SCC towards its original levels was observed in experiments with dDAVP application after the hormone removal ($P < 0.01$).

It is concluded that dDAVP stimulates Na^+ transport through the frog skin despite its lacking pressoric activity. Thus, the natriferic activity of vasopressin is related to its antidiuretic rather than pressoric activity. Maximum increase in the sodium transport following dDAVP application was delayed and more protracted as compared to the effect of LVP.

Key words: Lysine-vasopressin —dDAVP — Short-circuit current — Frog skin

Introduction

The synthetic analogue of vasopressin, 1-deamino-8-D-arginine-vasopressin (dDAVP), has a protracted activity while having practically no pressoric effects when applied systemically (Zaoral et al. 1967). In comparative studies of structure and function of LVP and dDAVP it has been found that the building-up of the cortico-papillary osmotic gradient in the kidney and stimulation of both the Na-K-ATPase (Michajlovskij et al. 1980) and the adenylate cyclase activities

(Dousa et al. 1975) in the renal medulla are predominantly bound to the antidiuretic activity of the vasopressin molecule. On the other hand, the stimulatory effect of vasopressin on ACTH secretion (Andersson et al. 1979; László et al. 1983) and its ability to increase growth hormone secreation (M. Vigaš, B. Lichardus, unpublished results) may rather be ascribed to its pressoric activity since dDAVP does not stimulate the secretion of these hormones. Aimed at extending comparative studies of LVP and dDAVP the effect of the hormones on sodium transport in the frog skin were studied in the present work.

It was found that sodium transport as determined by short-circuit current measurements was stimulated by equimolar doses of both LVP and dDAVP. The maximal increase in the sodium transport following dDAVP application was delayed and the effect was protracted as compared to that of LVP.

Materials and Methods

Animals and experimental setup: The experiments were performed on isolated abdominal skin of the frog *Rana temporaria* of both sexes ($n = 24$). The animals were kept at 4 °C in containers continually perfused with tap water. They were transferred to the laboratory (room temperature) and immediately used for the experiment. The spine was dissected and the abdominal skin removed. For the estimation of the active sodium transport through the skin the method of the short-circuit current was used (Ussing and Zerahn 1951). The isolated skin was stretched between two half-chambers filled with recirculating aerated Ringer's solution for Amphibia. Both compartments of the chamber were filled with Ringer's solution of identical concentration (in mmol . l^{-1}): NaCl 112; KCl 1.9; $CaCl_2$ 1.1; $NaHCO_3$ 2.4; pH = 8—8.3. Each compartment of the chamber had a volume of 15 ml. The active surface of the skin was 1.33 cm^2. The skin transepithelian potential difference (U) was measured continually by a pair of Ag-AgCl electrodes mounted into agar bridges. The tips of these potential measuring electrodes were placed as close to the skin surface as possible. Another pair of Ag-AgCl electrodes, principially of the same construction as those used to measure the potential, were placed at a distance of 2 cm from the skin. The latter pair of electrodes was used for short-circuiting the skin potential difference. The electric current measured under condition of short-circuiting the skin potential to zero (the short-circuit current: SCC) is considered to be a reliable reflection of the active ion (mainly Na^+) transport through the skin epithelium (Ussing and Zerahn 1951). Recordings of the short-circuit current were performed at 15 min intervals. The electric D.C. resistance (R_s) was expressed as the ratio of the transepithelial potential difference and the short-circuit current at zero transepithelial potential. The skin potential difference was measured by a high input resistance device (TESLA MULTIMETER BM 518) and the short-circuit current by a sensitive microampermeter (TESLA). The voltage necessary to short-circuit the skin potential was supplied by dry batteries, the circuit being regulated manually with a 10 kohm potentiometer. Hormone application: After an equilibration period of 1—2 h to stabilize the electric parameters of the skin the hormones tested were applied into the Ringer's solution bath at the inner surface of the skin (corium) in two different final concentrations: LVP 9.5×10^{-8} mol . l^{-1}, dDAVP 9.4×10^{-8} mol . l^{-1} lower concentration; LVP 18.9×10^{-8} mol . l^{-1}, dDAVP 18.7×10^{-8} mol . l^{-1} higher concentration. After reaching maximal stimulation of the transport, the hormone was washed out from the chamber by the same volume of fresh Ringer's solution after an additional 15 min interval. The washing was repeated with the first volume being discarded, and the electric parameters of the frog skin were recorded during the next 2 hours. The results were statistically evaluated using Student's t-test. The experimental groups comprised 6 animals each, the results were expressed as means ± S.E.

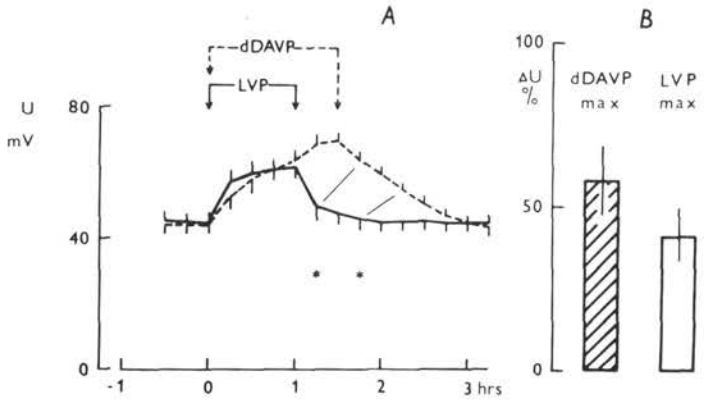

Fig. 1. Effects of lower dose of hormones upon the transepithelial potential difference (U). Time course of the effects of 9.4×10^{-8} mol . l^{-1} dDAVP (— — — —) and 9.5×10^{-8} mol . l^{-1} LVP (————) upon the electric parameters of sodium transport expressed in actual values (*A*) and in % of respective control values (*B*). The hormones were applied at 0 min to the inside bath solution. Fifteen minutes after having reached maximum stimulation of the transport the hormones were washed out from the chamber by the same volume of fresh Ringer's solution. Asterisks denote statistical significance ($P < 0.05 - P < 0.01$). Bars indicate the respective values of parameters which were compared and found statistically significant.

Results

Lower doses of the hormones: The application of dDAVP (final concentration 9.4×10^{-8} mol . l^{-1}) or LVP (9.5×10^{-8} mol . l^{-1}) resulted in similar values of maximal increase in the skin potential difference, short-circuit current and electric D.C. resistance, respectively (Figs. 1, 2 and 3). However, the maximal increase in bath the potential difference and the short-circuit current were delayed by about 30 min after the application of dDAVP. Also, significant differences in the parameters under investigation were found between the groups following the removal of the hormones. A more prolonged stimulation of the skin by dDAVP was observed following the removal of the hormone as compared to LVP. No change in the skin resistance was found after dDAVP application or dDAVP removal, while the application of LVP led to a decrease in the maximal electric direct current (D.C.) resistance ($P < 0.005$); following LVP removal the resistance restored to basal levels (Fig. 3).

Higher doses of the hormones: Contrary to lower doses of the hormones the maximal increase in both the skin potential and the short-circuit current occurred more rapidly following the higher LVP concentration (18.9×10^{-8} mol . l^{-1}) than following an equimolar concentration of dDAVP (18.7×10^{-8} mol . l^{-1}). There was

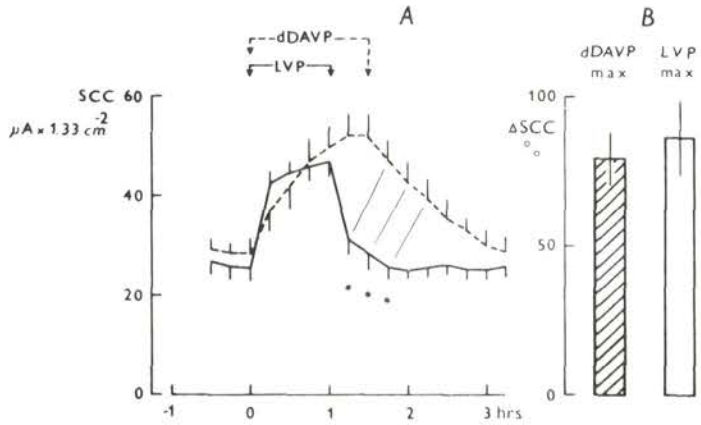

Fig. 2. Effects of lower dose of hormones upon the short-circuit current (SCC). (For symbols see Fig. 1; $P<0.05 - P<0.001$).

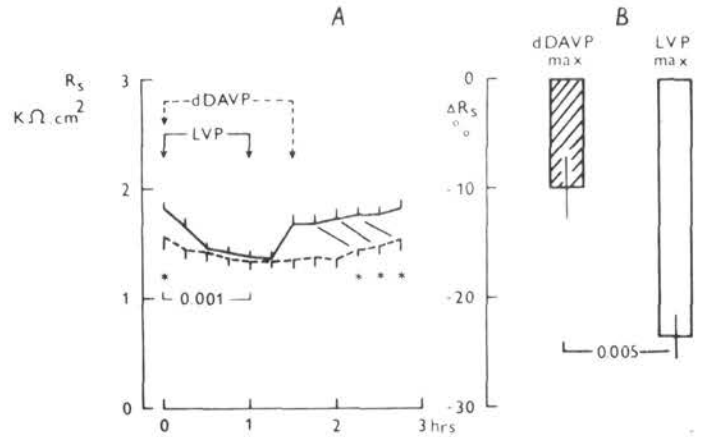

Fig. 3. Effects of lower dose of hormones upon the transepithelial D.C. resistance (R_s). (——P—— statistical significance of the parameter in maximum as compared to its basal level; other symbols are the same as in Fig. 1; $P<0.05 - P<0.02$).

no difference in the magnitude of the stimulation obtained by the two hormones. As with the lower doses, a protracted stimulation by dDAVP occurred following the removal of the hormones from the bathing solution (Fig. 4 and 5). These changes occurred under conditions of unchanged skin D.C. resistance after the application of dDAVP. No differences in the resistance were found between the groups or in the groups after the application of removal of dDAVP (Fig. 6). LVP application on the contrary decreased the transepithelial skin D.C. resistance, after both the lower ($P<0.001$), (Fig. 3) and the higher dose ($P<0.05$), (Fig. 6), as

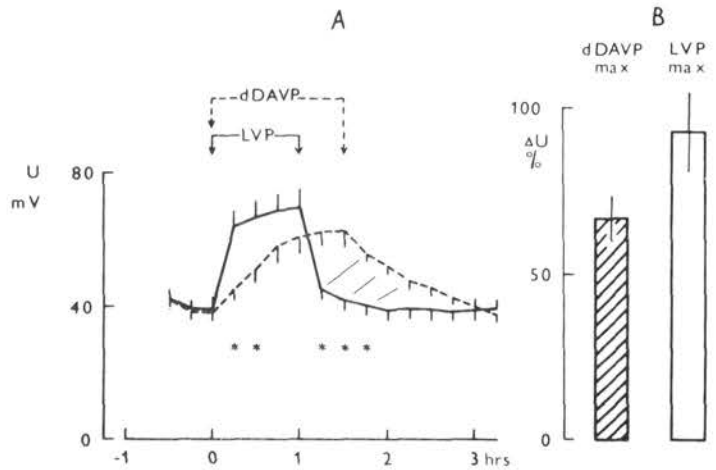

Fig. 4. Effects of higher dose of hormones upon the transepithelial potential difference (U). Time course of the effects of 18.7×10^{-8} mol . l^{-1} dDAVP (— — — —) and 18.9×10^{-8} mol . l^{-1} LVP (————) upon the electric parameters of sodium transport.) (For other symbols, see Fig. 1; $P<0.05 - P<0.01$).

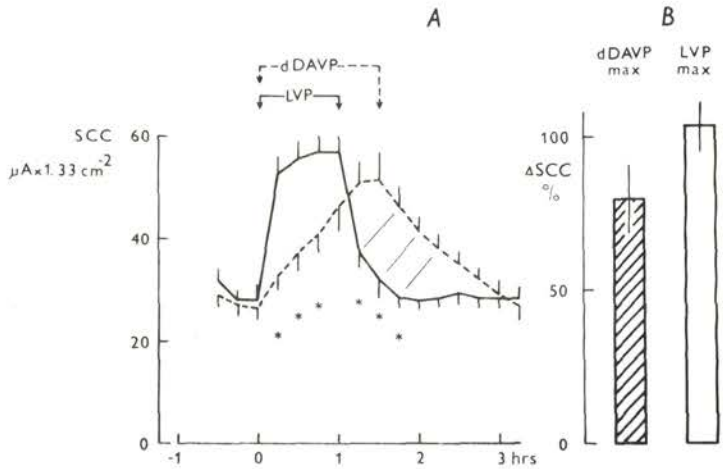

Fig. 5. Effects of higher dose of hormones upon the short circuit current (SCC) (For symbols see Fig. 1; $P<0.05 - P<0.001$).

shown by comparing the maximal decrease to the initial level of the epithelial resistance.

Both the doses of dDAVP resulted in identical increase in short-circuit current and potential difference. However, the higher dose of LVP as compared to the lower one exerted a significantly more pronounced increase the short-circuit current (Table 1, Figs. 2 and 5).

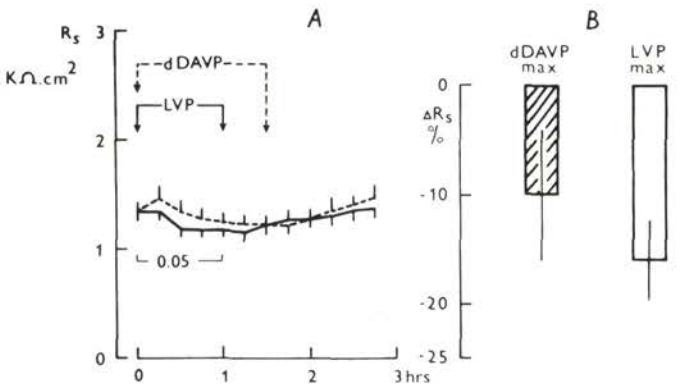

Fig. 6. Effects of higher dose of hormones upon the transepithelial D.C. resistance (R_s). (——P—— see Fig. 3). (*For symbols see Fig. 1*).

Discussion

As confirmed by our results both LVP and dDAVP, a synthetic analogue of vasopressin, stimulated the active transport processes through the frog skin epithelium. Following the application of corresponding doses of LVP and dDAVP, both the stimulation of the transepthelial skin potential and that of the short-circuited current were practically equal. However, there was a delay in stimulation following the application of dDAVP and the time to reach maximum stimulation was prolonged by about 30 minutes. It is well documented that dDAVP has a protracted antidiuretic effect when applied systemically (Vávra et al. 1968; Némethová and Lichardus 1974; Marek et al. 1978). This may be result of its longer biological half-life (t 1/2 for dDAVP in the fast and slow phase are 7.8 min and 75.5 min respectively; t 1/2 for LVP in the fast and slow phase are 2.5 min and 14.5 min respectively; Edwards et al. 1973). This may reflect the fact that the new molecule also proves to be more resistant to enzymatic cleavage (Zaoral et al. 1967). This quality of dDAVP may also be responsible for its protracted effect observed in our in vitro experiments.

The ratio of antidiuretic to pressoric activities of LVP equals 1. As compared to LVP, this ratio is higher for dDAVP (about 79 at lower doses of dDAVP and 2500—4500 at higher doses; Vávra et al. 1968). Thus at antidiuretic doses the pressoric effect of dDAVP may be considered as minimal. The substitution of D-arginine for L-arginine in the vasopressin molecule minimizes the pressoric activity of dDAVP (Zaoral et al. 1967). Based on these and our present data showing equal stimulation of Na^+ transport by both LVP and dDAVP, it may be concluded that the stimulatory effect of vasopressin on the active transepithelial sodium transport is bound to the antidiuretic activity of the vasopressin molecule.

Table 1. The effect of a lower and a higher dose of lysine-vasopressin (LVP) on the short-circuit current (SCC). The hormone was applied to the skin at zero time interval and its effect was registered in 15 min intervals for 1 hour. The values are means ± S.E. $n = 6$.

	SCC $\mu A \times 1.33\ cm^{-2}$					
LVP 9.5×10^{-8} mol . l^{-1}	25.5 ± 1.96	25.3 ± 1.80	42.5 ± 2.83	44.6 ± 2.81	46.0 ± 3.08	47.0 ± 3.15
LVP 18.9×10^{-8} mol . l^{-1}	28.1 ± 1.62	28.0 ± 1.55	53.6 ± 3.21	56.1 ± 3.14	57.1 ± 3.20	57.8 ± 3.13
P<	NS	NS	0.05	0.05	0.05	0.05
Time in min	−15	0	15	30	45	60

The previously documented hydroosmotic activity of vasopressin on amphibian bladder (Hays and Leaf 1962; Carasso et al. 1966) does not seem to be pertinent to our present experiments. As shown by isolated cells of toad bladder (Eggena 1979) vasopressin does not show a significant hydroosmotic action. In addition, dDAVP has proven a poor hydroosmotic activity when tested on the frog bladder (Barth et al. 1975). All the above experiments were performed under conditions of different outside and inside concentrations of bathing Ringer's solutions. Thus, hydroosmotic activity of the hormones under investigation, if present at all, could not have been expected to influence substantially the effects described in our experiments.

According to a hypothesis of Koefoed-Johnsen und Ussing (1958), the frog skin epithelium may be considered as a two membrane system, the outer membrane being selectively permeable for sodium ions and the inner one comprising Na/K exchange mechanism.

As shown by others, vasopressin enables sodium to enter epithelial cells across the outer membrane and an increased intracellular Na^+ concentration stimulates the Na/K exchange pump in the basolateral membrane (Andreoli and Schaffer 1976). The receptors for vasopressin are sited on the inner (basolateral) plasma membrane of the epithelial cells. Hormone — receptor interaction stimulated the formation of intracellular cAMP by an action on the membrane-bound enzyme adenylate cyclase (Orloff et al. 1962).

In microelectrode studies, vasopressin decreased the resistance of the outer membrane of the epithelial cells with small change in the inner membrane (Civan and Frazier 1968; Rawlins et al. 1970); Nagel 1978).

In our experiments with LVP and a synthetic analogue of AVP, dDAVP, a relatively small decrease in transepithelial resistance was found; this may be due to opposite alterations of the outer and inner membrane resistance. Similar small decrease in transepithelial resistance was described by Nagel (1978) who found that the decreased outer membrane resistance after vasopressin application was

partially counteracted by an increase in the inner membrane resistance. Since in our experiments the decrease in the transepithelial resistance following dDAVP application were small or even absent we conclude that any probable decreases in the outer membrane resistance was counteracted by a compensatory increase in the inner membrane resistance. On the other hand, the decrease in D.C. resistance after LVP application may have been due to a decrease in the outer membrane resistance (Nagel 1978).

Finally, it is noteworthy that the increase of short-circuit current exerted by two different doses of LVP was found dose-dependent whereas the effect of two different doses of dDAVP was identical. This finding suggests that the sodium transport mechanisms of the frog skin may be more sensitive to the stimulation by dDAVP than by LVP.

In conclusion, 1) dDAVP, a synthetic analogue of vasopressin, when studied in an in vitro system shows a similar protracted effect as observed previously in vivo; 2) since the pressoric activity of dDAVP is very small compared to natural vasopressin, the stimulatory effect of vasopressin upon the active sodium transport through epithelia could be ascribed to the antidiuretic activity of the vasopressin molecule; 3) a possible decrease in the outer membrane resistance was probably counteracted by the same increase in the inner membrane resistance since small or no changes in transepithelial resistance were found after dDAVP application; 4) the sodium transport mechanisms seem to be more sensitive to the stimulation with dDAVP than with LVP.

Acknowledgement. The authors wish to thank Dr. K. Janáček for his critical comments during the preparation of the manuscript.

References

Andersson K. E., Arner B., Nender P., Mulder J. L. (1979): Effect of 8-lysine-vasopressin and synthetic analogues on release of ACTH. Acta Endocr. (Kbh.) **69**, 640—648

Andreoli T. E., Schafer J. A. (1976): Mass transport across cell membranes: The effects of antidiuretic hormones on water and solute flows in epithelia. Annu. Rev. Physiol. **39**, 451—500

Barth T., Le Bars N.-C., Roy Ch., Jard S. (1975): Hydroosmotic activity of "carba" analogues of oxytocin and (8-arginine) vasopressin on frog (Rana esculenta) bladder. Eur. J. Pharmacol. **32**, 214—220

Carasso N., Gavard P., Bourguet J., Jard S. (1966): Rôle du flux net d'eau dans les modifications ultrastructurales de la vessie de Grenouilles stimulée par l'ocytocine. J. Microscopie **5**, 519—522

Civan M. M., Frazier H. S. (1968): The site of the stimulatory action of vasopressin on sodium transport in toad bladder. J. Gen. Physiol. **51**, 589—605

Dousa T. H., Hui Y. F. S., Barnes L. D. (1975): Renal medullary adenylate cyclase in rats with hypothalamic diabetes in sipidus. Endocrinology **97**, 802—807

Edwards C. R. W., Kitau M. J., Chard T., Besser G. M. (1973): Vasopressin analogue dDAVP in diabetes insipidus: Clinical and laboratory studies. Brit. Med. J. **3**, 375

Eggena P. (1979): Effect of vasopressin on the permeability to water of isolated toad bladeer epithelial cells INSERM **85**, 301—310

Hays R. M., Leaf A. (1962): Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. J. Gen. Physiol. **45**, 905—919

Koefoed-Johnsen V., Ussing H. H. (1958): The nature of the frog skin potential. Acta Physiol. Scand. **42**, 298—308

László F. A., Janáky T., Julesz J., Kárteszi M., Makara G. B., Stark E. (1983): Effects of arginine-8-vasopressin and 1-deamino-8-D-arginine-vasopressin on ACTH secretion in rats. Endocr. Exper. **17**, 133—136

Marek J., Loutocký A., Pacovský, V., Zaoral M. (1978): Ten-year experience with dDAVP in treatment of diabetes insipidus. Endokrinologie **72**, 188—194

Michajlovskij N., Ponec J., Džurba A., Lichardus B. (1980): (Na-K)ATPase activity and corticopapillary osmotic gradient in the kidney of rats with hereditary hypothalamic diabetes insipidus after administration of 1-deamino-8-D-arginine-vasopressin (dDAVP). Bratisl. lek. Listy **73**, 741—748 (in Slovak)

Nagel W. (1978): Effects of antidiuretic hormone upon electrical potential and resistance of apical and basolateral membranes of frog skin. J. Membrane Biol. **42**, 99—122

Némethová V., Lichardus B. (1974): Clinical experience with dDAVP (1-deamino-8-D-arginin-vasopressin) in the treatment of diabetes insipidus in children. Endokrinologie **63**, 137—141

Orloff J., Handler J. S., Preston A. S. (1962): The similarity of effects of vasopressin, adenosine 3′, 5′-phosphate (cyclic AMP) and theophylline on toad bladder. J. Clin. Invest. **41**, 702—709

Rawlins F., Mateau L., Fragachan F., Whittenbury G. (1970): Isolated toad skin epithelium: Transport characteristics. Pflügers Arch. **316**, 64—80

Ussing H. H., Zerahn K. (1951): Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol. Scand. **23**, 110—127

Vávra J., Machová A., Holeček V., Cort J. H., Zaoral M., Šorm F. (1968): Effect of a synthetic analogue of vasopressin in animals and in patients with diabetes insipidus. Lancet **1**, 948—952

Zaoral M., Kolc J., Šorm F. (1967): Amino acids and peptides LXXXI. Synthesis of 1-deamino-8-D-gamma-aminobutyrine-vasopressin, 1-deamino-8-D-lysine-vasopressin, 1-deamino-8-D-arginine-vasopressin. Coll. Czechoslov. Chem. Commun. **32**, 1250—1257

Received December 16, 1983/Accepted February 24, 1984