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HYDROXY ANALOGUES OF OXYTOCIN AND OF LYSINE-VASOPRESSIN

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1 Synthetic analogues of oxytocin and of lysine-vasopressin with an hydroxyl group in either the L or D configuration replacing the primary amino group have been tested for biological activity.

2 [1-(L-2-Hydroxy-3-mercaptopropanoic acid)] oxytocin ([L-Hmp[1]]oxytocin) was 1.5 to 2 times more potent than oxytocin on the rat uterus *in situ*, the rat mammary strip and the rat mammary gland *in situ* and 3 times more potent on the rat isolated uterus.

3 The pressor activity of [1-(L-2-hydroxy-3-mercaptopropanoic acid)-8-lysine]vasopressin ([L-Hmp[1], Lys[8]] vasopressin) was 2.2 and the antidiuretic activity 2.1 times that of lysine-vasopressin.

4 The [D-Hmp[1]] analogues of oxytocin and vasopressin were much less potent than the [L-Hmp[1]] analogues.

5 The responses to oxytocin and its hydroxy analogues *in vivo* were qualitatively indistinguishable but the pressor and antidiuretic responses to the hydroxy analogues of lysine-vasopressin were prolonged compared with those to the parent hormone.

6 The hydroxy analogues of oxytocin and lysine-vasopressin were not inactivated by pregnancy plasma oxytocinase.

7 The results are discussed in relation to the importance of the primary amino group for the biological activity and metabolism of the neurohypophysial hormones.

Introduction

The hormones of the mammalian neurohypophysis, oxytocin and vasopressin, satisfy two different structural requirements in their non-covalent binding to tissue receptors and to their carrier proteins, the neurophysins. This is exemplified by synthetic deamino analogues of oxytocin (Hope, Murti & du Vigneaud, 1962; Chan & du Vigneaud, 1962), of lysine-vasopressin (Kimbrough, Cash, Branda, Chan & du Vigneaud, 1963) and of arginine-vasopressin (Huguenin & Boissonnas, 1966) which are more potent than their parent hormones in many of their pharmacological activities but are not bound to neurophysin (Stouffer, Hope & du Vigneaud, 1963; Hollenberg & Hope, 1967). In these analogues, the primary amino (NH_2) group of the hemicystine residue in position 1 of oxytocin and the vasopressins is replaced by a hydrogen atom. The absolute requirement of the NH_2 group for binding to neurophysin is consistent with the view that an electrostatic bond is formed. In an attempt to define the binding characteristics of the NH_2 group more closely, Wälti & Hope (1972; 1974) synthesized two analogues, [1-(L-2-hydroxy-3-mercaptopropanoic acid)] oxytocin ([L-Hmp[1]]oxytocin) and its diastereoisomer ([D-Hmp[1]] oxytocin) in which this group is replaced by an approximately isoteric but uncharged hydroxyl group. Neither analogue was bound to neurophysin thus confirming that binding occurs through electrostatic, rather than hydrogen bonds (Hope & Wälti, 1971). [L-Hmp[1]] oxytocin was found to be about three times as active as oxytocin on the rat isolated uterus and ten times as active on rat blood pressure (Hope & Wälti, 1974). The surprisingly high activity on the isolated uterus led to an investigation of activity on the rat uterus *in situ* and on the rat mammary gland both isolated and *in situ*. The results are described in this paper. Since this work was carried out [L-Hmp[1]] oxytocin has been synthesized by two other groups (Manning, Lowbridge, Haldar & Sawyer, 1976; Stahl & Walter, 1977) in the course of their studies of structure-activity relationships and a wide spectrum of pharmacological activities has now been described for this analogue.

The disproportionately high pressor activity of

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[L-Hmp^1] oxytocin prompted the synthesis of the corresponding analogue of lysine-vasopressin [1-(L-2-hydroxy-3-mercaptopropanoic acid)-8-lysine] vasopressin ([L-Hmp^1, Lys^8] vasopressin) and its diastereoisomer ([D-Hmp^1, Lys^8] vasopressin), neither of which is bound to neurophysin (Wälti & Hope, 1975). Results of assays of the pressor and antidiuretic activities of these analogues are also given in this paper. Substitution of the NH_2 group of lysine-vasopressin by an OH group has been found not only to enhance both activities but also to prolong the duration of action whereas no such effect was observed with the oxytocin analogues. An attempt is made to explain this difference in terms of the metabolism of the parent hormones. In this connection, the susceptibility of the hydroxy analogues to hydrolysis by plasma oxytocinase (Tuppy, 1968) has been investigated.

Methods

Assay preparations

Rat isolated uterus Oestrus was induced in a virgin rat by intramuscular injection of 70 μg oestradiol benzoate 18 h before the assay and confirmed by examination of a vaginal smear. A uterine horn was removed and suspended in a 5 ml organ bath at 29 to 32°C containing Munsick's solution (Munsick, 1960), as formulated by Stürmer (1968), without magnesium. The composition was as follows: (mM) NaCl 113, KCl 6.1, $CaCl_2$ 0.5, NaH_2PO_4 0.18, Na_2HPO_4 0.82, $NaHCO_3$ 30.5, glucose, 2.8. The solution was gassed with a mixture of 95% O_2 and 5% CO_2. In some experiments tachyphylaxis developed during the first few responses to oxytocin. This was corrected, without inducing spontaneous uterine activity, by increasing the concentration of $CaCl_2$ to 0.625 mM. An initial tension of 1 to 2 g was applied to the uterine horn and contractions were recorded isotonically with a photoelectric transducer and potentiometric recorder (Servoscribe RE 511). Oxytocin or an hydroxy analogue was added to the organ bath at intervals of 5 min and the bath washed out as soon as the contraction had reached its peak.

Rat uterus in situ Intraluminal fluid pressure was recorded from a uterine horn in an anaesthetized rat in pro-oestrus, and uterine activity measured with a digital integrator, according to the method of Bisset, Haldar & Lewin (1966). Injections were given intravenously.

Isolated strip of rat mammary gland The method was based on that of Rydén & Sjöholm (1962). Strips were usually obtained from lactating rats which had been used for the assay of milk-ejecting activity. A strip 3 cm × 0.5 cm × 0.5 cm was cut radially from the teat of an abdominal or inguinal mammary gland and stored overnight at 4°C in Tyrode solution without magnesium (mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.6). It was suspended in a 5 ml organ bath containing the same solution, gassed with a mixture of 95% O_2 and 5% CO_2. Tension was recorded isometrically with a Statham universal transducing cell (Model UL3) and micro-scale accessory (Model UL5) connected with the same recorder as that used for the isolated uterus. An initial tension of 400 to 500 mg was applied and the tissue allowed to equilibrate for 1 to 2 h before the start of an assay. The organ bath was washed out by overflow 2 min after the addition of oxytocin or hydroxy analogue. To avoid tachyphylaxis, an interval of at least 8 min was allowed between doses with further changes of bath fluid as required to restore the resting tension.

Mammary gland in situ Milk-ejection pressure was recorded from a cannulated teat duct in an anaesthetized lactating rat by the method of Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva (1967). The retrograde arterial route of injection was used in two assays and the intravenous route in six. As no difference was observed, the results have been combined.

Rat blood pressure Pressor activity was assayed in male rats weighing 250 g anaesthetized with urethane (0.3 g in 1.25 ml 0.9% NaCl solution, i.p.). The α-adrenoceptor blocking agent, phenoxybenzamine, 1 mg, was injected intraperitoneally 1 h before the start of the assay. The hormones and analogues were injected intravenously through a cannula in the external jugular vein and blood pressure was recorded from a carotid artery with a strain gauge transducer and potentiometric recorder.

Rat urine flow Antidiuretic activity was assayed in male rats weighing 250 g, anaesthetized with ethanol, in which a constant fluid load equivalent to 8% of the body weight was maintained by intravenous infusion of 2 to 3% ethanol in isotonic glucose-saline solution. Urine flow was measured by means of a drop counter connected with a staircase integrator and potentiometric recorder (see Clark & Rocha e Silva, 1967). For quantitative assays, the antidiuretic response was measured in terms of intensity, i.e. the reduction in urine flow from the 2nd to the 6th min, inclusive, after the injection expressed as a percentage of the control flow before the injection (Bisset, 1962). The duration of the response was measured as the number of minutes required for the urine flow to return to 90% of the pre-injection level.

Design of assays

A (2 and 2) dose assay with at least 3, and usually 4 or more, blocks was carried out on all preparations and the results were submitted to analysis of variance according to the procedure described by Schild (1942). Results of repeated assays were combined to give an estimate of weighted mean potency with 95% fiducial limits ($P = 0.05$) by the method described in the British Pharmacopoeia (1968). Only those assays were included in which there was no significant ($P = 0.05$) deviation from parallelism between the log dose-response curves for standard and test.

The hydroxy analogues of oxytocin were assayed against synthetic oxytocin (Syntocinon) and biological activity was expressed in u/mg. The International Standard was considered to be unsuitable for assaying hydroxy analogues of lysine-vasopressin since it contains arginine-vasopressin and the profiles of the antidiuretic responses to these two hormones differ considerably (see Figure 3). Commercially available lysine-vasopressin was also rejected because this is itself assayed against the International Standard. Therefore, the hydroxy analogues were assayed against a sample of lysine-vasopressin synthesized in the same laboratory and the results expressed in terms of relative potency rather than absolute units of activity. The weighted mean potency of this sample of lysine-vasopressin, estimated from six assays of pressor activity against the Third International Standard for oxytocin and vasopressin (Bangham & Mussett, 1958), was 168 (160 to 176) u/mg.

Incubation of peptides with pregnancy plasma

Plasma was obtained from women during the 36th week of pregnancy. Oxytocin, 20 mu, or [L-Hmp^1] oxytocin, 20 ng, was incubated with 0.2 ml plasma and lysine-vasopressin, 100 ng, or [L-Hmp^1, Lys^8] vasopressin, 50 ng, with 0.1 ml plasma for 120 min at 37°C. The total volume of the incubation mixture was 2 ml: all dilutions were made with 0.9% NaCl solution (pH 7.4). For incubations at pH 4, acetic acid was added to the incubation mixture to give a final concentration of 1%. At the end of the incubation, the samples at pH 7.4 were similarly acidified. All samples were then placed in a boiling water bath for 5 min, cooled and neutralized with 1N Na_2CO_3 before assay.

Materials

Synthetic lysine-vasopressin was prepared in the Department of Pharmacology, Oxford by the procedure of Bondanszky, Meienhofer & du Vigneaud (1960). This peptide and the hydroxy analogues of oxytocin and lysine-vasopressin prepared in the same laboratory (see Introduction) were in analytically pure form (microanalysis and amino-acid analysis). Other peptides used were synthetic oxytocin (Syntocinon, Sandoz); [1-(*N*-carbamoyl-hemicystine-2-*O*-methyltyrosine)] oxytocin (carbamoyl-methyloxytocin: Chimiak, Eisler, Jošt & Rudinger, 1968) and 1-deamino-8-D-arginine-vasopressin (desmopressin: Ferring Pharmaceuticals Ltd.).

Results

Hydroxy analogues of oxytocin

The results of assays of both hydroxy analogues of oxytocin are shown in Table 1 (reference 8). The pharmacological activities of oxytocin and deamino-oxytocin and of [L-Hmp^1] oxytocin prepared by other workers are included for comparison (references 1 to 7). In all our assays, the analogues produced responses that were indistinguishable qualitatively from those to oxytocin. The analogues gave the same maximum response as oxytocin: there was no difference in the duration of action *in vivo* and the analogues did not potentiate or inhibit the response to subsequent doses of oxytocin.

[L-Hmp^1] oxytocin had a potency of 874 (814 to 939) u/mg on the rat uterus *in situ*, 799 (748 to 854) u/mg on the isolated rat mammary strip and 815 (777 to 854) u/mg on the rat mammary gland *in situ* (milk-ejecting activity). Overlap of the 95% fiducial limits indicates that these estimates are not significantly different. [D-Hmp^1] oxytocin had a potency of only 2 to 15% that of [L-Hmp^1] oxytocin on the rat uterus, isolated or *in situ*, and on the mammary gland *in situ*.

Figure 1 illustrates an experiment on the rat isolated uterus to test the effect of a specific antagonist of oxytocin [1-(*N*-carbamoyl-hemicystine-2-*O*-methyltyrosine)] oxytocin (carbamoyl-methyloxytocin; Bisset, Clark, Krejčí, Poláček & Rudinger, 1970) on the response to [L-Hmp^1]oxytocin. Graded responses of similar magnitude were obtained with 0.2, 0.4 and 0.8 mu oxytocin (a, 1, 2 and 3) and 0.2, 0.4 and 0.8 ng [L-Hmp^1] oxytocin (b, 4, 5 and 6). The largest dose of each was repeated after increasing doses of 0.1, 0.2, 0.4 and 0.8 μg carbamoyl-methyloxytocin (C). This analogue produced reversible inhibition of the response to [L-Hmp^1] oxytocin which showed a close parallelism to its inhibition of the response to oxytocin. In contrast, there was no inhibition of the responses to acetylcholine (c, 7, 8 and 9).

In the experiments with pregnancy plasma, incubation of oxytocin at pH 7.4 resulted in 88% loss of milk-ejecting activity but there was no detectable loss of activity with oxytocin at pH 4 or with [L-Hmp^1] oxytocin at either pH.

Table 1 Biological activities (u/mg) of oxytocin, deamino-oxytocin and hydroxy analogues

	Rat uterus			*Mammary gland*						
	Isolated		*In situ*	*Rat*		*Rabbit*	*Chicken*	*Rat*	*Rat*	
	No Mg^{2+}	*0.5 to 1.0 mM Mg^{2+}*		*Isolated strip*	*In situ*	*In situ*	*blood pressure*	*blood pressure*	*urine flow*	*Refs*
Oxytocin	546 ± 18[1]	420 ± 10[1]	468 ± 35[1]	533 ± 62[2]		410 ± 16[3]	507 ± 23[3]	3.1 ± 0.1[3]	2.7 ± 0.2[3]	1,2,3
Deamino-oxytocin	551 ± 17[1]	476 ± 15[1]	487 ± 41[1]			400 ± 8[3]	733 ± 23[3]	1.1 ± 0.1[3]	15.0 ± 2.0[3]	1,3
amorphous										
crystalline	803 ± 36[4]					541 ± 13[4]	975 ± 24[4]	1.44 ± 0.06[4]	19[4]	4
[L-Hmp[1]]	1607							32		5
oxytocin										
	1275 ± 51	868 ± 101		363 ± 8	694 ± 43			14.7 ± 0.3	16.6 ± 1.3	6
	1542 ± 18						1778 ± 25	32.7 ± 0.4	40.3 ± 2.4	7
	1641		874	799	815					8
	(1546–1743)		(814–939)	(748–854)	(777–854)					
[D-Hmp[1]]	27		73		121					8
oxytocin	(21–37)		(36–110)		(106–140)					

The values quoted from references 1 to 7 are means ± s.e. Those given in the present paper (8) for [L-Hmp[1]] oxytocin analogue are weighted mean potencies with 95% fiducial limits, estimated from at least 6 assays, and for [D-Hmp[1]] oxytocin, the potencies with 95% fiducial limits for single assays. References: 1. Chan & Kelley (1967); 2. Polaček, Krejčí & Rudinger (1967); 3. Chan & du Vigneaud (1962); 4. Ferrier, Jarvis & du Vigneaud (1965); 5. Hope & Wälti (1971; 1974); Wälti & Hope (1972); 6. Manning, Lowbridge, Haldar & Sawyer (1976); 7. Stahl & Walter (1977); 8. Present paper.

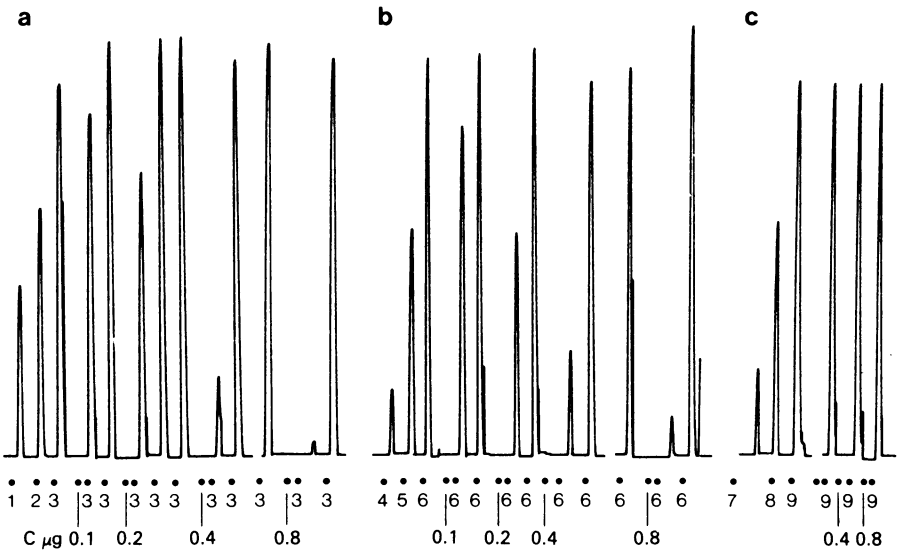

Figure 1 Rat isolated uterus. Isotonic contractions: 5 ml organ bath. The effect of an oxytocin antagonist, carbamoyl-methyloxytocin (C), on the responses to (a) oxytocin (1 = 0.2, 2 = 0.4, 3 = 0.8 mu); (b) [L-Hmp^1] oxytocin (4 = 0.2, 5 = 0.4, 6 = 0.8 ng); and (c) acetylcholine (7 = 0.05, 8 = 0.1, and 9 = 0.2 øg). The antagonist was added to the organ bath 1 min before oxytocin, the L-Hmp^1 analogue or acetylcholine which were tested at 5 min intervals. The organ bath was washed out as soon as a contraction had reached its peak.

Hydroxy analogues of lysine-vasopressin

The hydroxy analogues of lysine-vasopressin produced relatively prolonged pressor and antidiuretic responses with profiles distinctly different from those obtained with the parent hormone. Figure 2 illustrates an experiment on rat blood pressure in which an injection of either 30 ng lysine-vasopressin (LV) or 15 ng [L-Hmp^1, Lys^8] vasopressin (L-Hmp^1-LV) was given regularly at intervals of 15 min. These doses were shown to give rises in blood pressure of the same amplitude. A typical response to lysine-vasopressin is shown in the first trace (a); several previous responses had been obtained at intervals of 15 min with no evidence of tachyphylaxis. The injection of 30 ng produced a rise in blood pressure of 48 mm Hg (a 1): after 5 min (a 2) this rise had decreased by 33% and 10 min after the injection (a 3) the blood pressure had returned to the baseline. The first injection of the analogue (b) produced a rise of 47 mmHg but this decreased by only 14% after 5 and 81% after 10 min. With repeated injections of the analogue the prolongation of the pressor response was accentuated, the blood pressure failed to return to the baseline even after 15 min and the amplitude was slightly reduced. This is exemplified by the response shown in (d). The baseline was raised above the initial control level (cf. a) when the injection was given: a rise of 43 mmHg was reduced by only 6% after 5 and 40% after 10 min. Repeated injections of the analogue altered the response to subsequent injections of lysine-vasopressin. The amplitude of the first response to lysine-vasopressin given after the analogue (e) was reduced and the duration prolonged compared with the initial response (a): the rise was now 40 mm and this was decreased by only 14% after 5 and 77% after 10 min. After repeated injections of Lysine-vasopressin the baseline decreased and the profile resembled that originally obtained (cf. a and f).

Figure 3 illustrates typical antidiuretic responses to lysine-vasopressin (LV), [L-Hmp^1, Lys^8] vasopressin (L-Hmp^1-LV) and also to arginine-vasopressin (AV) and its analogue, desmopressin (DES), which is notable for the long duration of its antidiuretic response. With both desmopressin and [L-Hmp^1, Lys^8] vasopressin, the initial antidiuretic response for the first 10 min was similar to that observed with the parent hormone but the urine flow then reached a plateau and, after a slight decline, there was a slow progressive recovery to the pre-injection level. It should be noted that the response to arginine-vasopressin is more prolonged than that to lysine-vasopressin. The structural modifications in the two analogues produced comparable prolongations of the responses to their respective parent hormones. A comparison was made between the durations of the antidiuretic responses to all doses of lysine-vasopressin and [L-Hmp^1, Lys^8] vasopressin which produced re-

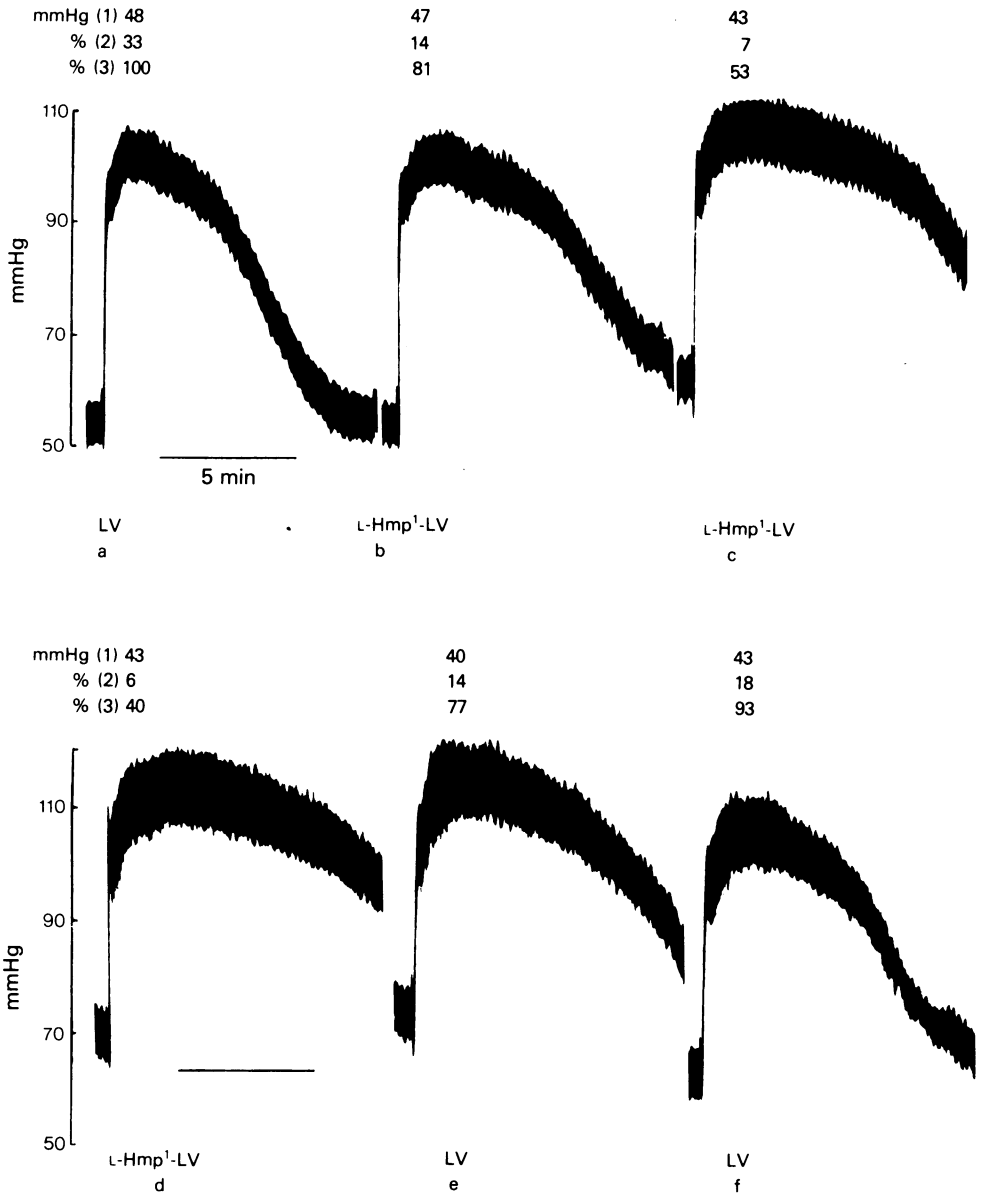

Figure 2 Rat blood pressure. An intravenous injection of 30 ng lysine-vasopressin (LV) or 15 ng [L-Hmp^1, Lys^8] vasopressin (L-Hmp^1-LV) was given every 15 min. Each trace shows the pressor response recorded during the period of 10 min following the injection. Traces (a), (b) and (c), and (d) and (e) are consecutive: between (c) and (d) an injection of L-Hmp^1-LV was given and between (e) and (f) four injections of LV were given. The figures above each trace show: (1) the maximum rise in blood pressure (mmHg); (2) and (3) the % reduction in this rise 5 min and 10 min, respectively, after the injection.

sponses of similar intensity within the range 40 to 60% (i.e. the percentage reduction in flow between the 2nd to 6th min after injection; see Methods). The mean response to 29 doses of 100 to 400 pg lysine-vasopressin was 49.5% ± 0.90 (s.e.) and the mean duration 7.6 min ± 0.11 (s.e.). For 23 doses of 75 to 200 pg [L-Hmp^1, Lys^8] vasopressin, the mean response was 50.7% ± 1.25 and the mean duration 12.7

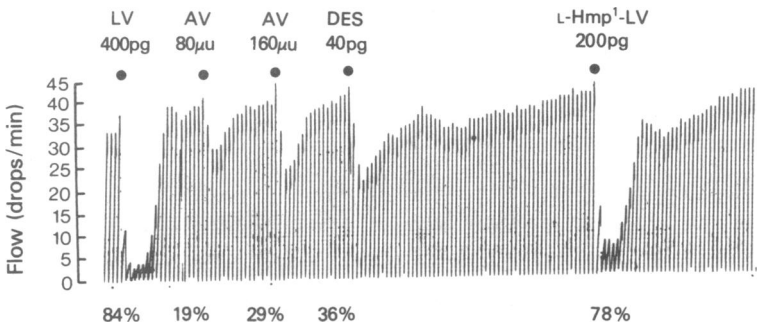

Figure 3 Comparison of the antidiuretic response to [L-Hmp^1, Lys^8] vasopressin (L-Hmp^1-LV), lysine-vasopressin (LV), arginine-vasopressin (AV) and 1-deamino-8-D-arginine-vasopressin (desmopressin: DES) in the rat. Urine flow was recorded from a rat under ethanol anaesthesia with a constant water load equivalent to 8% of its body weight. Each vertical line in the record represents urine flow measured as number of drops in one minute (1 drop = 4.3 μl). The dots above the record indicate the minute at the beginning of which an intravenous injection of LV, AV, DES or L-Hmp^1-LV was made. The values below the record show the % reduction in urine flow during the 5 min period following the minute in which the injection was made, expressed as a % of the control flow immediately preceding the injection. Note the prolonged responses to DES and L-Hmp^1-LV.

min ± 0.65. For the same intensity the hydroxy analogue produced a significantly ($P = <0.001$) longer duration of action.

The qualitative difference between the responses to lysine-vasopressin and the hydroxy analogues made it difficult to obtain valid quantitative assays of potency. However, by using relatively small doses of analogue and allowing a sufficient interval of time between doses to allow full or practically full recovery, it was possible to obtain valid four-point assays (see Methods). The results of 5 assays of pressor activity gave a weighted mean relative potency of [L-Hmp^1, Lys^8] vasopressin with respect to lysine-vasopressin of 2.23 (2.12 to 2.36). For antidiuretic activity the relative potency derived from 6 assays was 2.08 (1.99 to 2.18). Since these limits just overlap, the two potencies do not differ significantly. For [D-Hmp^1, Lys^8] vasopressin single assays gave a relative potency of 0.01 for both pressor and antidiuretic activities.

In the experiments with pregnancy plasma, incubation of lysine vasopressin at pH 7.4 resulted in >96% loss of antidiuretic activity. Incubation of [L-Hmp^1, Lys^8] vasopressin at pH 7.4 led to a loss of only 4% and no loss was detectable with either lysine-vasopressin or [L-Hmp^1, Lys^8] vasopressin at pH 4.

Discussion

Substitution of an hydroxyl (OH) for the primary amino (NH_2) group in oxytocin enhanced all the biological activities tested but not to the same extent. There was no statistically significant difference between the values which we obtained for the potency of [L-Hmp^1] oxytocin when assayed against oxytocin on the rat uterus *in situ*, the rat mammary strip and the rat mammary gland *in situ*. The potency, which ranged from 799 to 874 u/mg, was 1.5 to 2.0 times that of oxytocin assayed against the International Standard on the same preparations in the rat or rabbit (Table 1). In contrast with our results, Manning *et al.* (1976) found that the analogue was twice as potent on the rat mammary gland *in situ* as on the isolated strip. No explanation can be offered for this discrepancy but it may be noted that their value of 363 u/mg for activity on the isolated strip is the only one so far reported which is lower than that of oxytocin. The potency of [L-Hmp^1] oxytocin on the rat isolated uterus was 1641 u/mg or three times that of oxytocin. This disproportionately high activity appears to result from the absence of Mg^{2+} from the Munsick's solution in the bath fluid. Other workers using the same experimental conditions have obtained a potency of 1607 u/mg (Hope & Wälti, 1971; 1974; Wälti & Hope, 1972): 1275 u/mg (Manning *et al.*, 1976) and 1542 u/mg (Stahl & Walter, 1977). However, for the rat isolated uterus in the presence of 0.5 M Mg^{2+}, Manning *et al.* (1976) obtained a potency of 868 u/mg: this is close to the potency which we obtained on the rat uterus *in situ* and on the rat mammary gland both isolated and *in situ*. Magnesium is known to potentiate the response of the isolated uterus to neurohypophysial hormones and synthetic analogues although the degree of potentiation is variable (see Munsick, 1968). Chan & Kelley (1967) found a remarkably close agreement between the potencies of oxytocin, deamino-oxytocin, deamino-deoxy-oxytocin and 4-decarboxamido-oxytocin assayed against the International Standard on the rat uterus *in situ* and on the rat isolated uterus in the presence of 2 mEq/l Mg^{2+}. However, the potency on the rat iso-

lated uterus without Mg^{2+} was much lower for deamino-deoxy-oxytocin and for 4-decarboxamido-oxytocin but higher for deamino-oxytocin. [L-Hmp^1] oxytocin resembles deamino-oxytocin but the enhancement of activity on the isolated uterus in the absence of Mg^{2+} is relatively greater. A possible interpretation of these findings is that Mg^{2+} potentiates the uterine response to oxytocin more than that to its hydroxy and deamino-analogues. In the presence of Mg^{2+}, either in the organ bath or *in vivo*, their potency in relation to oxytocin would be reduced and, in its absence, enhanced. It is more difficult to account for the exceptionally high avian depressor activity of [L-Hmp^1] oxytocin reported by Stahl & Walter (1977).

Although too little of the antagonist, *N*-carbamoyl-*O*-methyl oxytocin, was available to determine pA_2 values (Arunlakshana & Schild, 1959), the experiment illustrated in Figure 1 provides some evidence that [L-Hmp^1] oxytocin acts on the same tissue receptor as oxytocin on the isolated uterus. The greater potency of the analogue may reflect an increase of affinity for the receptor or efficacy, either of which may in turn be affected by the presence or absence of Mg^{2+}. An important factor in determining the potency of oxytocin could be the charge on its NH_2 group. At physiological pH, this group is charged to the extent of about 10%. The hydroxyl group is isosteric with the NH_2 group but uncharged. This suggests that, if the NH_2 group in oxytocin is involved at all in binding to the receptor, it is active in the uncharged form. Substitution of a hydroxyl group produces greater enhancement of potency than deletion of the NH_2 group. This may be not only because the hydroxyl group is uncharged but also because its presence confers a better steric configuration for binding to the receptor. The importance of steric influences is apparent from our finding that the [D-Hmp^1] analogues of oxytocin and lysine vasopressin possessed only a small fraction of the activities of the L isomers.

An interesting difference between the deamino and [L-Hmp^1] analogues of both oxytocin and lysine-vasopressin lies in the ratio of pressor and antidiuretic activities. Hope & Wälti (1974) first reported the relatively high pressor potency of [L-Hmp^1] oxytocin which they found to be 10 times that of oxytocin (Table 1). Stahl & Walter (1977) confirmed this finding, and found that the antidiuretic activity was equally high. Manning *et al.* (1976) also found that the pressor and antidiuretic activities were approximately equal but only 5 times those of oxytocin. We found no significant difference between the pressor and antidiuretic activities of [L-Hmp^1, Lys^8] vasopressin which was about twice as potent as the parent hormone. The hydroxy analogue of arginine-vasopressin has now been synthesized and this shows a ratio of antidiuretic to pressor activity of 0.9 (Lowbridge, Manning, Haldar & Sawyer, 1977). In contrast the deamino analogues of oxytocin and the vasopressins reveal an enhancement of antidiuretic and reduction of pressor activity. The ratio of antidiuretic to pressor activity is 15 for deamino-oxytocin (Chan & du Vigneaud, 1962), 2.4 for deamino-lysine vasopressin (Kimbrough *et al.*, 1963) and 3.5 for deamino-arginine-vasopressin (Huguenin & Boissonnas, 1966). In the analogue, desmopressin (1-deamino-8-D-arginine vasopressin,), the additional substitution of the D-isomer of arginine raises the ratio further to 79 (Vavrá, Machová, Holocek, Cort, Zaoral, & Šorm, 1968) or to 2000 according to Sawyer, Acosta, Balaspiri, Judd & Manning (1974a).

A striking observation with the hydroxy analogues of lysine vasopressin was that both the pressor and antidiuretic responses were prolonged compared with responses of equal intensity to the parent hormone. In this respect [L-Hmp^1, Lys^8] vasopressin showed a remarkable similarity to desmopressin (Figure 3). It has been clearly shown that the prolongation of the antidiuretic response to desmopressin is attributable solely to deamination and is uninfluenced by substitution of D-arginine in position 8: in fact, the antidiuretic responses to 1 deamino-arginine vasopressin and desmopressin are equally prolonged (Sawyer *et al.*, 1974a; Sawyer, Acosta & Manning, 1974b). A prolonged antidiuretic response to deamino-lysine vasopressin has also been reported (Chan, 1965). Substitution of the terminal NH_2 group by an OH group appears to be as effective as its deletion in prolonging antidiuretic activity. However, whereas there is no significant difference between the pressor and antidiuretic activities of hydroxy-lysine-vasopressin, substitution of the D-isomer of arginine in desmopressin has the effect of almost completely suppressing pressor activity.

Sawyer *et al.* (1974b) have suggested that the prolonged antidiuretic response to deaminated vasopressin results from resistance to enzymatic degradation. One enzyme system which has been implicated in the metabolism of neurohypophysial hormones *in vitro* involves an aminopeptidase detected in beef liver which splits the cysteine-tyrosine bond in oxytocin and vasopressin: an essential preliminary step is reduction of the disulphide (S–S) bond by an enzyme which is probably identical with glutathione-insulin transhydrogenase (Rychlík, 1964). Pregnancy plasma oxytocinase is an aminopeptidase which splits the cysteine-tyrosine bond without preliminary reduction of the S–S bond (Tuppy, 1968). Golubow, Chan & du Vigneaud, (1963) showed that oxytocinase did not inactivate deamino-oxytocin and our results have shown a similar resistance of [L-Hmp^1] oxytocin and [L-Hmp^1, Lys^8] vasopressin to this enzyme. Prolongation of the responses to vasopressin by deamination

or by substitution of the NH_2 group by an OH group suggests that the NH_2 group is important for metabolism. Its importance for the metabolism of oxytocin is more difficult to assess. Barth, Krejčí, Vaněčková, Jošt & Rychlík (1974) reported a prolonged uterine response *in vivo* to deamino oxytocin and to deamino-carba analogues in which the S–S bond is replaced by $S–CH_2$ or $CH_2–CH_2$. Other workers have reported no prolongation of the uterine response to deamino-oxytocin (Chan & Kelley, 1967; Barth, Hütter, Pliška & Šorm, 1969) or the antidiuretic response to deamino-oxytocin (Pliška, Rudinger, Dousa & Cort, 1968; Barth *et al.*, 1969; Sawyer *et al.*, 1974b) or to MeCys'- and D-Cys'-oxytocin (Rychlík, 1964). We observed no prolongation of the responses of the uterus or mammary gland *in vivo* to [L-Hmp^1] oxytocin. Most of the evidence therefore suggests that the aminopeptidase system is not important for the metabolism of oxytocin.

Pliška *et al.* (1968) suggested that some part of the oxytocin molecule other than the S–S bond or primary NH_2 group was involved. A carboxypeptidase has been detected in rat uterus which inactivates oxytocin and deamino-oxytocin by splitting the bond between leucine in position 8 and glycinamide in position 9 but does not inactivate lysine, or arginine, vasopressin (Glass, Dubois, Schwartz, & Walter, 1970; Walter & Shlank, 1971). A similar enzyme in rat kidney has been found to inactivate oxytocin, deamino-oxytocin and deamino-dicarba-oxytocin but not arginine-vasopressin (Koida, Glass, Schwartz & Walter, 1971). These findings emphasise the importance of the NH_2 group for the metabolism of vasopressin and that of the leucine-glycinamide bond for the metabolism of oxytocin.

Walter and his co-workers have isolated a post-proline cleaving enzyme from the human uterus (Walter, Shlank, Glass, Schwartz & Karenyi, 1971) and from the liver of various species (Yoshimoto, Orlowski & Walter, 1977) which inactivates oxytocin and vasopressin *in vitro* at equal rates by splitting the bond between proline and the adjacent amino acid in position 8, whether this is an L- or D-isomer. If the prolonged duration of action of hydroxy and deamino analogues of vasopressin is due to resistance to enzymatic degradation, the results suggest that *in vivo* under physiological conditions, the amino peptidase system is of greater importance than the post-proline cleaving enzyme.

Finally it is prudent to bear in mind, as has been pointed out by Pliška & Rudinger (1976) that peripheral inactivation processes are only one factor in determining the prolongation of action of analogues and that kinetic factors such as diffusion in the receptor compartment may be of greater importance.

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