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# THE BIOLOGICAL ACTIVITY OF A NEW ANALOGUE OF OXYTOCIN IN WHICH THE TYROSYL GROUP IS REPLACED BY PHENYLALANYL

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A new synthetic analogue of oxytocin with a phenylalanyl group instead of a tyrosyl group has been investigated. The most prominent biological properties of this peptide were its oxytocic activity on the rat or cat uterus *in vivo* and on milk-ejection pressure in the rabbit. Another remarkable feature was the striking difference in pressor activity measured in spinal cats and in anaesthetized rats. The presence of the phenylalanyl group in the molecule in the above-mentioned position did not increase the antidiuretic potency in unanaesthetized rats. The authors propose a nomenclature for the peptides related to oxytocin and vasopressin.

As chemical and biological work on peptides related to oxytocin and vasopressin progresses, the need for a simple and clear nomenclature in this field becomes increasingly greater. We should therefore like to propose a nomenclature along the lines of that already in use for polypeptides of the hypertensin type (see, for example, Rittel, Iselin, Kappeler, Riniker, and Schwyzer, 1957). The amino-acid groups in the oxytocin molecule would be numbered as follows:

1 2 3 4 5 6 7 8 9
CyS-Tyr-Ileu-GluNH$_2$-AspNH$_2$-CyS-Pro-Leu-GlyNH$_2$

The number of the group which the new amino-acid replaces would be indicated as a superscript to the new group. The analogue dealt with in this paper would therefore be named phenylalanyl$^2$-oxytocin. The oxytocin analogues which have so far been investigated (Rudinger, Honzl, and Zaoral, 1956; Boissonnas, Guttmann, Jaquenoud, Waller, Konzett, and Berde, 1956a; Berde, Doepfner, and Konzett, 1957; Katsoyannis, 1957; Ressler and du Vigneaud, 1957; Ressler and Rachele, 1958) would then be specified as follows: phenylalanyl$^3$-oxytocin (this is also known as oxypressin or the P-analogue); leucyl$^3$-oxytocin (also known as the L-analogue); valyl$^3$-oxytocin (also known as valyl-oxytocin or the V-analogue); glutaminyl$^5$-oxytocin (also known as the G-analogue); and isoglutaminyl$^4$-oxytocin (also known as isoglutamine isomer of oxytocin or isoglutamine-oxytocin). With this nomenclature, the vasopressins could also be described as oxytocin analogues, namely arginine-vasopressin as phenylalanyl$^3$-arginyl$^8$-oxytocin and lysine-vasopressin as phenylalanyl$^3$-lysyl$^8$-oxytocin. In the case of the synthetic higher homologues the term " homo " would precede the new amino-acid group and would have as superscripts the numbers of those two groups between which the new one has been inserted, for example, homotyrosyl$^{2:3}$-oxytocin (Guttmann, Jaquenoud, Boissonnas, Konzett, and Berde, 1957; Konzett, 1957). In the present paper the above nomenclature will be used.

Recent work in this laboratory (Boissonnas *et al.*, 1956a; Berde *et al.*, 1957; Berde and Cerletti, 1957; Guttmann *et al.*, 1957; Konzett, 1957; Berde and Cerletti, 1958) and elsewhere (Katsoyannis, 1957; Ressler and du Vigneaud, 1957; Ressler and Rachele, 1958) on the biological activity of synthetic analogues of oxytocin has revealed that relatively small chemical changes in the oxytocin molecule may lead to a decrease, increase, or modification of its characteristic effects.

If, for example, the asparaginyl group in the oxytocin molecule is replaced by a glutaminyl group (glutaminyl$^5$-oxytocin), the physiological and pharmacological actions peculiar to the oxytocic hormone of the pituitary are absent (Berde *et al.*, 1957). The same holds true if an isoglutaminyl group is substituted for the glutaminyl group (isoglutaminyl$^4$ - oxytocin) (Ressler and du Vigneaud, 1957; Ressler and Rachele, 1958). The biological activity of the

oxytocin analogue which contains a leucyl group instead of the isoleucyl group (leucyl$^3$-oxytocin) is rather modest (Berde *et al.*, 1957). Nevertheless, this compound exerts all the typical actions of oxytocin, albeit on a reduced scale.

However, replacing the isoleucyl group of the oxytocin molecule by a valyl group (valyl$^3$-oxytocin) brings a striking increase of the *in vivo* activity on the uterus and on the mammary gland, together with a decrease of the pressor and antidiuretic potency (Boissonnas *et al.*, 1956a; Berde *et al.*, 1957). This means that the uterus-stimulating and milk-ejecting effects of valyl$^3$-oxytocin are even more specific than those of the natural oxytocic hormone.

The cyclic octapeptide possessing a phenylalanyl group in the position where oxytocin contains an isoleucyl group (phenylalanyl$^3$-oxytocin) is interesting because this compound is, chemically, an intermediate between oxytocin and vasopressin. This fact is also borne out by pharmacological studies (Berde *et al.*, 1957; Katsoyannis, 1957): both the qualities of oxytocin (uterus-stimulating, milk-ejecting, and avian depressor activity) and of vasopressin (pressor and antidiuretic activity) are exhibited with remarkably high potency.

Phenylalanine is found in the vasopressin obtained both from cattle and pigs. It could therefore be assumed that the phenylalanyl group *per se* is mainly responsible for the effects peculiar to vasopressin. The substitution of a phenylalanyl group for any amino-acid group in oxytocin would then cause biological properties to shift towards those of vasopressin. On the other hand, it could be argued that, in order to exert such a decisive influence on the pharmacological character of the cyclic octapeptide, the phenylalanyl group would have to occupy the same position which it occupies in the vasopressin molecule, where it is located in the ring between the tyrosyl and the glutaminyl group. The pharmacological study of a synthetic analogue of oxytocin with a phenylalanyl group in some other position was therefore of considerable interest.

Such an analogue of oxytocin with a phenylalanyl group substituted for the tyrosyl group, phenylalanyl$^2$-oxytocin (Table I), has recently been synthesized in our Department of Pharmaceutical Chemistry by P. A. Jaquenoud in 1958 using the same method of synthesis as previously used for the synthesis of oxytocin (Boissonnas, Guttmann, Jaquenoud, and Waller, 1955): To obtain the cyclic octapeptide, 20 mg. of the corresponding nonapeptide (Boissonnas, Guttmann, Jaquenoud, and Waller, 1956b) was dissolved in 5 ml. of liquid ammonia, and reduced with sodium (du Vigneaud, Ressler, Swan, Roberts, and Katsoyannis, 1954). Ammonium chloride (5 mg.) was added; the ammonia was evaporated and the residue added to 100 ml. of water, the *p*H adjusted to 6.8, $CO_2$-free air bubbled through the solution for 2 hr., and the *p*H adjusted to 4.5. Saline was used for further dilution.

TABLE I

THE SEQUENCE OF THE AMINO ACIDS IN OXYTOCIN AND IN PHENYLALANYL$^2$-OXYTOCIN

CyS = L-cysteinyl; Tyr = L-tyrosyl; Ileu = L-isoleucyl; Glu($NH_2$) = L-glutaminyl; Asp($NH_2$) = L-asparaginyl; Pro = L-prolyl; Leu = L-leucyl; Gly($NH_2$) = glycinamide; Phe = phenylalanyl.

| | |
|---|---|
| Synthetic oxytocin | CyS-Tyr-Ileu-Glu($NH_2$)-Asp($NH_2$)-CyS-Pro-Leu-Gly($NH_2$) |
| Phenylalanyl$^2$-oxytocin | CyS-Phe-Ileu-Glu($NH_2$)-Asp($NH_2$)-CyS-Pro-Leu-Gly($NH_2$) |

## METHODS

Six tests were used to detect and measure pharmacological effects peculiar to oxytocin. These included the rat uterus *in vitro* (Holton, 1948) and *in vivo* (Berde *et al.*, 1957), as well as the cat uterus *in vivo* (Berde *et al.*, 1957) for the uterus-stimulating (oxytocic) activity; the mammary gland of lactating rabbits (Van Dyke, Adamsons, and Engel, 1955; Berde and Cerletti, 1957) for the milk-ejecting potency; the blood pressure of chickens (Coon, 1939; Thompson, 1944) for the avian depressor effect; and the diuresis of saline-loaded rats (Lipschitz, Hadidian, and Kerpcsar, 1943) for the diuretic effect.

Four tests were used to demonstrate and measure biological activities characteristic of vasopressin. These were the blood pressure of spinal cats and the blood pressure of rats pre-treated with dibenamine and heparin, according to Dekanski (1952), for the pressor potency; the rabbit ileum *in vitro* for the effect on the smooth muscle of the intestine; and the diuresis of water-loaded rats (Burn, 1931; Burn, Finney, and Goodwin, 1950) for the antidiuretic quality. In the first three of these tests, the effectiveness of the unknown preparation was estimated by bracketing doses of the unknown with doses of the standard. The results obtained did not permit accurate determination of the limits of error. To measure the antidiuretic activity a four-point assay design (Schild, 1942) was employed.

The Third International Standard for Posterior Pituitary (the Third International Standard for Oxytocic, Vasopressor, and Antidiuretic Substances) was used for comparison in all but one test. In the diuresis experiments on saline-loaded rats, synthetic oxytocin, also

known as Syntocinon (Boissonnas *et al.*, 1955; Konzett, Berde, and Cerletti, 1956), was used for this purpose.

## RESULTS

The results are summarized in Table II. The values obtained with the two conventional methods for the bioassay of oxytocin (isolated rat uterus and chicken blood pressure) differed,

TABLE II

ACTIVITY IN VARIOUS TESTS OF 1 ML. OF SOLUTION OF PHENYLALANYL$^3$-OXYTOCIN IN UNITS OF THE INTERNATIONAL STANDARD (THIRD INTERNATIONAL STANDARD FOR OXYTOCIC, VASOPRESSOR, AND ANTIDIURETIC SUBSTANCES)

Synthetic oxytocin was used in the diuretic test as the basis of comparison. Standard errors shown in parentheses.

| | |
|---|---|
| Isolated rat uterus | 1·6 (±0·16) |
| Chicken blood pressure | 2·6 (±0·24) |
| Isolated rabbit uterus | 1·6 |
| Rat uterus *in vivo* | 3·7 (±0·64) |
| Cat ,, ,, ,, | 10·7 (±2·2) |
| Milk-ejection pressure in rabbit | 6·2 (±0·64) |
| Pressor activity in spinal cat | 0·16 |
| ,, ,, ,, rat | 0·016 |
| Isolated rabbit intestine | 0·016 |
| Antidiuretic activity | 0·024 (±0·006) |
| Diuretic ,, | 10·7 (±6·7) |

whereas the activity on the isolated rat uterus and the isolated rabbit uterus was identical. The activities on the uterus *in vivo* of rats and cats were considerably higher than that on the rat uterus *in vitro*, as was the action on the milk-ejection pressure in the rabbit mammary gland.

There was a quite remarkable difference between the pressor activity on the spinal cat and the pressor activity on the anaesthetized rat. The potency on the isolated rabbit intestine resembled that on the blood pressure of the rat rather than that on the blood pressure of the spinal cat. The antidiuretic activity was low, whereas the diuretic activity was remarkably high.

It is quite clear from these findings that the potency of the new analogue, expressed in units of the International Standard for Posterior Pituitary, depends on the biological indicator used for the assay. As the relative activities in the different tests may be of special interest, these are given in Table III, the oxytocic effect on the rat uterus *in vitro* being taken arbitrarily as 100. To facilitate a comparison of the new analogue with oxytocin and some interesting oxytocin analogues which have been previously described, Table III also gives the corresponding relative values for synthetic oxytocin, for valyl$^3$-oxytocin, and for phenylalanyl$^3$-oxytocin, as established in this laboratory (Konzett *et al.*, 1956; Berde *et al.*, 1957).

TABLE III

RELATIVE ACTIVITIES IN VARIOUS TESTS OF SYNTHETIC OXYTOCIN AND OF SOME ANALOGUES OF OXYTOCIN

The potency of each compound as measured on the isolated rat uterus is arbitrarily taken as 100. Diuretic potency of synthetic oxytocin arbitrarily taken as 100 and used as standard of comparison for the diuretic activity of phenylalanyl$^3$-oxytocin.

| Test | Phenyl-alanyl$^3$-oxytocin | Synthetic Oxytocin | Valyl$^3$-oxytocin | Phenyl-alanyl$^3$-oxytocin |
|---|---|---|---|---|
| Isolated rat uterus | 100 | 100 | 100 | 100 |
| Chicken blood pressure | 165 | 101 | 114 | 81 |
| Isolated rabbit uterus | 100 | 100 | | |
| Rat uterus *in vivo* | 230 | 100 | 300 | |
| Cat ,, ,, ,, | 670 | 130 | 600 | 89 |
| Milk-ejection pressure in rabbit | 390 | 104 | 536 | 215 |
| Pressor activity in spinal cat | 10 | 0·9 | 0·5 | 19 |
| Pressor activity in rat | 1 | 1·1 | | |
| Antidiuretic activity | 1·5 | 1·2 | 1·4 | 104 |
| Diuretic activity | 670 | 100 | 71 | |

## DISCUSSION

Comparison of the relative potency of the new oxytocin analogue having a phenylalanyl group instead of the tyrosyl group with that of oxytocin and of other previously investigated analogues of oxytocin yields some remarkable facts.

The activity of phenylalanyl$^2$-oxytocin in the two conventional bioassays for oxytocin (the rat uterus *in vitro* and the chicken blood pressure) was discrepant, whereas the corresponding values for oxytocin, valyl$^3$-oxytocin and phenylalanyl$^3$-oxytocin were in good agreement. However, the cyclic disulphide ring of oxytocin (which has no prolyl - leucyl - glycinamide side - chain) also exhibited some discrepancy when assayed in the conventional way: it exerted an effect on the isolated rat uterus but none on the chicken blood pressure (Ressler, 1956). By contrast, the new analogue exerted a greater effect on the chicken blood pressure than on the isolated rat uterus.

The potency of phenylalanyl$^2$-oxytocin measured on the rat and cat uterus *in vivo* and on the rabbit mammary gland was definitely higher than when measured by the methods suggested by the pharmacopoeias (British Pharmacopoeia, 1958; United States Pharmacopeia XV, 1955) for the bioassay of oxytocin (rat uterus *in vitro*, chicken blood pressure). This type of discrepancy has already been observed with valyl$^3$-oxytocin, and to some degree with phenylalanyl$^3$-oxytocin (Boissonnas *et al.*, 1956a; Berde *et al.*, 1957) and

proved to be of practical importance, as the testing of valyl[3]-oxytocin on the human uterus *in vivo* also gave values higher than those obtained with the methods advised in the pharmacopoeias (Smyth, 1958). The same trend was found with leucyl[3]-oxytocin (Berde *et al.*, 1957), although this peptide was not tested on the rat uterus *in vivo*. As yet, no synthetic analogue of oxytocin has been found to show a discrepancy in the opposite sense, that is, an activity in the official bioassay methods higher than that on the uterus *in vivo* and on the lactating mammary gland. No explanation for this has yet been found.

The pressor activity of the new oxytocin analogue on spinal cats was much greater than that of oxytocin and valyl[3]-oxytocin, but smaller than that of phenylalanyl[3]-oxytocin. Thus, the presence of a phenylalanyl group in the cyclic part of the octapeptide increased the pressor activity of both compounds in spinal cats. It was very surprising that the activity of the new analogue on the blood pressure of anaesthetized rats was so much smaller (about 1/10) than that on the blood pressure of spinal cats. This was noteworthy, as the pressor activity of pituitary extracts from ox, cat, and rat was found to be practically identical by the rat and cat methods (Landgrebe, Macauley, and Waring, 1946). Furthermore, the results of our assays of the pressor potency of synthetic oxytocin and synthetic lysine-vasopressin on cats and rats showed close agreement.

Whereas the substitution of a phenylalanyl group for the isoleucyl group produced a tremendous increase of antidiuretic activity (from 1.2 to 104), the substitution of a phenylalanyl group for the tyrosyl group barely increased the antidiuretic activity (from 1.2 to 1.5). As far as water excretion was concerned, the presence of a phenylalanyl group instead of the tyrosyl group did not produce the appearance of vasopressin-like properties.

From the results reported it may be concluded that the position at which a certain amino-acid was substituted is very important for the biological activity of oxytocin analogues. The introduction of the same amino acid at various positions in the molecule may result in octapeptides with very marked differences in action, as a comparison of phenylalanyl[2]-oxytocin with phenylalanyl[3]-oxytocin showed. Whereas phenylalanyl[3] - oxytocin was biologically an intermediate between oxytocin and vasopressin, the oxytocin-like qualities of phenylalanyl[2]-oxytocin were paramount, especially *in vivo* (rat uterus, cat uterus, milk-ejection pressure).

It should, however, be borne in mind that in replacing one amino acid by another the actual chemical changes of the molecule may be small. Phenylalanyl[2]-oxytocin contains only one hydroxyl group less than oxytocin and valyl[3]-oxytocin contains only one $CH_3$ group less than oxytocin.

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