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QUANTITATIVE COMPARISON BETWEEN OXYTOCIN AND FOUR RELATED NEUROHYPOPHYSIAL PEPTIDES ON THE HUMAN UTERUS IN SITU

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Quantitative assays of four synthetic peptides related to oxytocin were carried out on the *post partum* human uterus *in vivo* by means of external tocography. The following activities (U/mg, means and standard errors) were found: isoleucine[8]-oxytocin 365 ± 70, asparagine[4]-oxytocin 150 ± 40, desamino[1]-oxytocin $1{,}030 \pm 300$, serine[4]-isoleucine[8]-oxytocin 335 ± 95. None of the conventional bioassay procedures proved to be fully reliable for predicting the oxytocic activity in man of peptides related to oxytocin. If the tocographically recorded effects of equipotent doses of the compounds are compared with those of oxytocin no qualitative differences can be observed.

The synthesis of numerous peptides related to the posterior pituitary hormones opened a wide field for pharmacological studies. The relationship between the chemical structures of these compounds and their biological properties has been elucidated extensively by Boissonnas, Guttman, Berde & Konzett (1961). Since then further structural analogues of oxytocin have been synthesized and submitted to pharmacological testing (Du Vigneaud, Winestock, Murti, Hope & Kimbrough, 1960; Jaquenoud & Boissonnas, 1961, 1962; Guttmann, Berde & Stürmer, 1962; Hope & Du Vigneaud, 1962; Huguenin & Boissonnas, personal communication, 1962; Berde & Saameli, communication to British Pharmacological Society, July 10–12, 1963). Assay methods have generally been used employing the following: rat isolated uterus and cat uterus *in situ* preparations, chicken blood pressure, mammary gland of the lactating rabbit, blood pressure in rats and antidiuresis in rats.

Only one of the compounds has so far been tested on the human uterus *in vivo*, namely valine[3]-oxytocin (Berde, Doepfner & Konzett, 1957). This substance gained special attention for several reasons, one being the more favourable relationship between uterotonic activity and undesirable pressor-antidiuretic effect (Smyth, 1958; Berde & Saameli, 1959; Cibils, Poseiro & Noriega-Guerra, 1961). The present study deals with four other structural analogues: isoleucine[8]-oxytocin, asparagine[4]-oxytocin, desamino[1]-oxytocin and serine[4]-isoleucine[8]-oxytocin. Knowledge of their activities on the human uterus *in vivo* was thought to be important, not only from a theoretical but also from a practical point of view, in particular with a therapeutic aim in mind or at least with a view to such a possibility.

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Continuous intra-uterine pressure recording on the pregnant uterus is generally regarded as the most reliable experimental method for assaying oxytocic drugs in human subjects because uterine activity can be evaluated in terms of tonus, intensity, frequency and duration of contractions (Alvarez & Caldeyro-Barcia, 1950 ; Embrey, 1958). The method, however, has several disadvantages:

(1) It involves a considerable amount of work on the part of physicians and nursing staff. Therefore under clinical conditions it can seldom be used on a sufficiently large scale to give statistically valid results.

(2) The dose-range of the substances being tested is strictly limited. The doses should be effective, but the necessity to avoid risk to mother and child is of paramount importance.

(3) Studies on the uterus near to term (not in labour) can only be performed when induction of labour is indicated, since labour may be induced by oxytocics even in small doses.

(4) The spontaneous activity of the parturient uterus is not constant but increases with progressing cervical dilatation.

None of these disadvantages is present with external tocography on the *post partum* uterus. This method can be used easily under clinical conditions without any hazard or even appreciable discomfort to the patients. The doses of the oxytocic to be tested can be varied within a wide range (relatively small doses, such as 100 mU of oxytocin intravenously, are usually effective whereas higher doses, such as 1,000 mU, do not cause undesirably strong effects). Studies on the *post partum* human uterus can be carried out at a fairly constant level of uterine activity, although this varies considerably from one patient to another and decreases rapidly during the first hr *post partum* but reaches a long-lasting stable level within 1.5 to 2 hr after delivery (Saameli, 1960). *Post partum* external tocography measures uterine contractions by means of an instrument fixed in a suitable manner on the abdominal wall. It responds to the forward rotation of the uterus during contractions as well as to changes in myometrial tone. In contrast to *ante partum* amniotic pressure recording, neither the basic tonus nor the intensity of the contractions can be recorded as absolute values, since both depend on the quality and thickness of the abdominal wall and the position of the uterus. However, the frequency of contractions and the duration of an elevated tonus can be evaluated with sufficient accuracy provided that the thickness of the abdominal wall does not exceed normal limits. The use of *post partum* external tocography in human subjects as a method for measuring pharmacological effects has been described previously by Myerscough & Schild (1955, 1958). Their method has been successfully used by others (Nixon & Smyth, 1957) and served in a modified form as a basis for the studies described here.

METHODS

The studies were carried out in sixty-six women (twenty-seven primiparae and thirty-nine multiparae) 3 to 7 hr after an uncomplicated vaginal delivery. According to the hospital routine the patients were given 0.1 mg of methylergometrine hydrogen maleate (Methergin) or 3 to 5 U of oxytocin (Syntocinon) intravenously at the time of delivery of the anterior

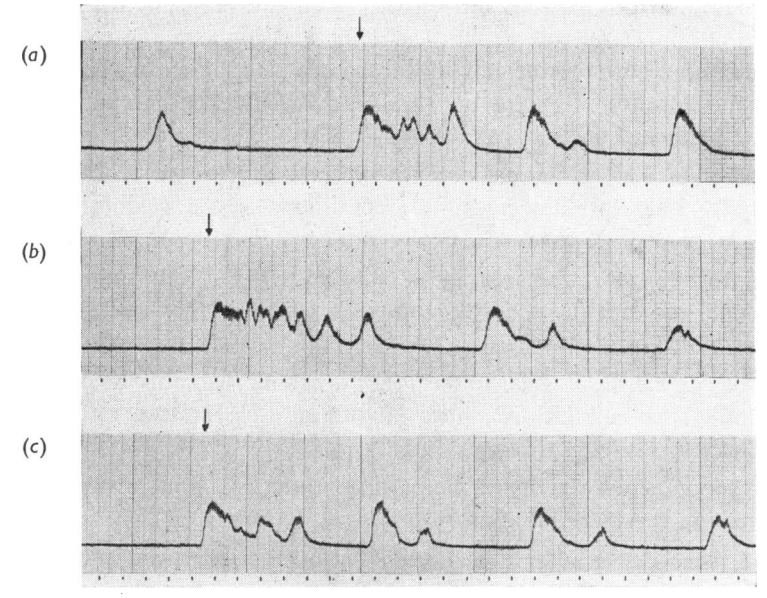

Fig. 1. A three-point matching assay on the human *post partum* uterus. External tocography. (*a*) and (*c*), at the arrows 0.15 U of desamino[1]-oxytocin intravenously; (*b*), at the arrow 0·6 U of synthetic oxytocin intravenously. The oxytocin : desamino[1]-oxytocin activity ratio is 4 : 1. Time marks in minutes.

shoulder or immediately after delivery of the baby. In order to suppress painful sensations and restlessness during the investigations most of the patients were given pethidine (50 to 100 mg, intramuscularly) often together with promethazine (50 mg, intramuscularly) 30 to 60 min before tocography started. Uterine contractility was recorded by a strain-gauge tocodynamometer (Smyth, 1957) connected to a potentiometric recorder (Honeywell-Brown), or by a photoelectric device recording on an electrocardiograph (Schwarzer). The tocometer was attached to the abdomen by an elastic belt over the upper part of the uterus. In some instances, it proved to be an advantage to use additional fixation in the form of adhesive plaster strips leading to the outside of both thighs in order to prevent it from slipping off. After spontaneous activity had been recorded for at least 30 min to establish a baseline, either oxytocin or one of the structural analogues was administered by rapid intravenous injection into an arm vein. Subsequent injections were done at intervals of 20 to 40 min provided the effect on contractility of the previous dose had diminished to the control level. In order to achieve comparable conditions all injections were done at the beginning of a spontaneous contraction (see Fig. 1). The doses used are shown in Table 1.

TABLE 1

DOSE RANGE FOR OXYTOCIN AND OXYTOCIN ANALOGUES APPLIED IN THREE-POINT MATCHING ASSAYS

1, rat isolated uterus preparation; 2, chicken blood pressure

Comparison, oxytocin versus	Dose range for	
	Oxytocin	Oxytocin analogue
Isoleucine[8]-oxytocin	0·066–0·62 U	0·05 –0·62 U[1]
Asparagine[4]-oxytocin	0·05 –0·5 U	0·075–1·2 U[2]
Desamino[1]-oxytocin	0·15 –0·6 U	0·05 –0·6 U[2]
Serine[4]-isoleucine[8]-oxytocin	0·125–0·5 μg	0·125–1·0 μg

The following experimental design was applied: the uterotonic activity of the oxytocin analogues being tested and that of synthetic oxytocin were compared in " three-point matching assays," a dose of one of the analogues being " bracketed " by two doses of oxytocin or *vice* versa. The two " bracketing " doses were identical, whereas the " bracketed " or intermediate dose was in a previously determined ratio to them. The dose ratios used are given in Table 2.

TABLE 2

COMPARISON OF THE UTEROTONIC EFFECTS OF OXYTOCIN ANALOGUES AND OXYTOCIN ON THE POST PARTUM HUMAN UTERUS *IN SITU*

The dose ratio is oxytocin : oxytocin analogue. Positive assays are those where the oxytocin analogue was stronger than oxytocin. * For this correction see Miller & Tainter (1944)

Peptide	Dose ratio	Assays: Positive	Assays: Negative	Positive (%)	Probit
Isoleucine[8]-oxytocin	0·63	5	0	95*	6·64
	1·0	3	2	60	5·25
	1·6	2	3	40	4·75
	2·5	0	5	5*	3·36
Asparagine[4]-oxytocin	0·25	5	0	95*	6·64
	0·5	4	1	80	5·84
	1·0	1·5	3·5	30	4·48
	2·0	0	5	5*	3·36
Desamino[1]-oxytocin	0·5	5	0	95*	6·64
	1·0	4·5	1·5	75	5·67
	2·0	1·5	3·5	30	4·48
	4·0	0	5	5*	3·36
Serine[4]-isoleucine[8]-oxytocin	0·25	5	0	95*	6·64
	0·5	4	1	80	5·84
	1·0	1·5	4·5	25	4·33
	2·0	0·5	4·5	10	3·72

Each dose ratio was tested in five (occasionally six) three-point matching assays, that is in five (or six) women. The interpretation of an assay was based on the determination whether the effect of the substance being tested was stronger or weaker than that of oxytocin, the prime criterion being the duration of the elevated tonus (Fig. 1). The result of a three-point matching assay was considered as " positive " if the effect of the structural analogue being tested was stronger than that of oxytocin and " negative " if it was weaker. In most instances there was no difficulty in deciding which effect was stronger. In seventeen out of eighty-two assays however, the reactions were so similar that any decision seemed purely arbitrary. In such cases the score was divided between the " positive " and the " negative " groups. Slight tachyphylaxis occurred in most instances and was taken into account in interpreting the results. For each ratio, oxytocin : oxytocin analogue, the relative frequency of the assays in which the analogue was stronger than oxytocin was calculated as a percentage and plotted against the logarithm of the corresponding dose ratio. Best fitting straight lines were drawn through each group of four points and the relative potency of the oxytocin analogues read at probit 5. The approximate standard error was calculated by the method of Miller & Tainter (1944).

RESULTS

The uterotonic effects of synthetic oxytocin and the oxytocin analogues were qualitatively indistinguishable. The quantitative differences are summarized in Table 2. Fig. 2 shows the straight lines which characterize the relationship between log dose and probit effect for each substance. By reading at probit 5 the following values were obtained (means and standard errors):

isoleucine[8]-oxytocin	1.26 ± 0.25
asparagine[4]-oxytocin	0.73 ± 0.20
desamino[1]-oxytocin	1.45 ± 0.42
serine[4]-isoleucine[8]-oxytocin	0.74 ± 0.21

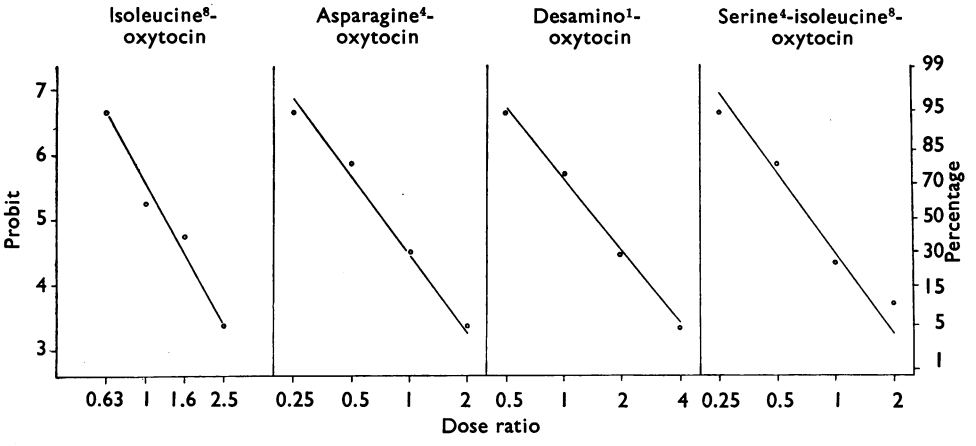

Fig. 2. The dose/response lines obtained by plotting the values in Table 2 in a log dose and probit effect coordinate system. The abscissa gives the oxytocin/oxytocin analogue dose ratio. The relative potency of the oxytocin analogues is read at probit 5.

Thus for isoleucine[8]-oxytocin, 1 U (activity estimated on the rat isolated uterus) had equal activity, assessed on the human uterus *in vivo*, to 1.26 ± 0.25 U of oxytocin ; or isoleucine[8]-oxytocin, compared unit for unit, is 1.26 ± 0.25 times more powerful than oxytocin. It is usual with substances related to oxytocin to express the activity in International Units/mg (U/mg). On the isolated rat uterus the activity of 1 mg of isoleucine[8]-oxytocin amounts to 289 U (Berde & Konzett, 1960). Its activity on the human uterus *in vivo* being, unit for unit, 1.26 ± 0.25 times greater than that of oxytocin, amounts therefore to 364 ± 72 U/mg. The corresponding values for all analogues tested in this study, and also for valine[3]-oxytocin which was assayed previously (Berde & Saameli, 1959), are given in Table 3.

TABLE 3

OXYTOCIN-LIKE ACTIVITIES OF NEUROHYPOPHYSIAL PEPTIDES IN CONVENTIONAL BIOASSAY PROCEDURES AND ON THE HUMAN UTERUS *IN SITU*

Values are means and standard errors. Bold type indicates nearest corresponding values for each peptide

Neurohypophysial peptides	Oxytocin-like activities (U/mg) on: Rat uterus (isolated)	Chicken blood pressure	Rabbit mammary gland	Cat uterus (*in situ*)	Human uterus (*in situ*)
Oxytocin	450	450	450	450	450
Valine[3]-oxytocin	59 ± 8	58 ± 4	207 ± 14	**226** ± 17	**277** ± 65
Isoleucine[8]-oxytocin	289 ± 21	498 ± 37	**328** ± 21	563 ± 74	**365** ± 70
Asparagine[4]-oxytocin	**108** ± 29	202 ± 12	300 ± 128	335 ± 75	**150** ± 40
Desamino[1]-oxytocin	360 ± 55	710 ± 60	440 ± 45	**900** ± 130	**1,030** ± 300
Serine[4]-isoleucine[8]-oxytocin	150 ± 12	**320** ± 15	300 ± 15	250 ± 40	**335** ± 95

DISCUSSION

Among the twenty-nine synthetic peptides closely related to oxytocin and known at present, only a few have shown considerable biological activity when tested by conventional bioassay methods. In the study described here all these active compounds except valine^{8}-oxytocin (Jaquenoud & Boissonnas, 1961) have been tested on the human uterus *in situ*.

The establishment of a relationship between the activities found on the human uterus *in vivo* and those obtained by animal bioassay would be of great interest. It was, of course, not expected that any of the conventional bioassay procedures for oxytocin would prove to be an exact indicator of potency in man. It is, however, conceivable that the receptors involved in one bioassay procedure might resemble more closely those connected with oxytocic activity on the human uterus than the receptors involved in other tests. This seemed all the more possible since the conventional assays for the oxytocin-like properties of the neurohypophysial hormones use such different structures and reactions as, for example, the contraction of the isolated uterine muscle of rats in oestrus, the relaxation of vascular smooth muscle in the chicken and the contraction of myo-epithelial elements of the lactating rabbit's mammary gland. Table 3 lists the activities of the synthetic peptides in question, as given in the literature (Boissonnas *et al.*, 1961), and in the last column the findings of the present study on the human uterus *in situ*. If the activities measured on the human uterus are compared with the values obtained in animal bioassays, the nearest corresponding values being emphasized by bold type, it is evident that none of the bioassay procedures included in this study proved to be fully reliable for predicting the oxytocic effect of a synthetic peptide of the neurohypophysial type on the human uterus *in vivo*. It seems, however, that some methods are more informative than others; the chicken blood pressure method, favoured by the pharmacopoeias for the standardization of oxytocin, and the rat uterus *in vitro* preparation, which is widely used probably because it is simple and cheap, are certainly not the best guides to predict the oxytocic activity of a neurohypophysial peptide in the human subject. The results obtained on the rabbit mammary gland *in situ* and the cat uterus *in situ* seem to be somewhat more dependable in this respect. Unfortunately, however, none of these conventional assay procedures for oxytocin is entirely reliable.

Valine^{3}-, isoleucine^{8}-, asparagine^{4}- and serine^{4}-isoleucine^{8}-oxytocin differ from oxytocin in their chemical structure in either one or two amino acid residues. These variations result in a decreased biological activity on the human uterus *in vivo* in comparison with oxytocin. Desamino^{1}-oxytocin, which was synthesized in 1960 (Du Vigneaud *et al.*, 1960; Hope & Du Vigneaud, 1962) and was produced on an industrial scale in 1962 (Huguenin & Boissonnas, personal communication) behaves differently. It differs from natural oxytocin in the absence of the free amino group of the cysteine residue in position 1. From the chemical point of view, this amino group is active and its loss might be expected to render the compound biologically inactive. However, this is not so. Its activity in different biological tests is even greater than that of naturally occurring oxytocin and amounts, on the *post partum* human uterus, to $1{,}030 \pm 300$ U/mg, which is about twice that of oxytocin. This

value agrees with unpublished results of Embrey (personal communication, 1963) who found desamino[1]-oxytocin to be 1.5 to 2 times more potent than oxytocin if compared unit for unit in pregnant, parturient and puerperal women.

It is well known that the serum of pregnant and early puerperal women contains an enzyme, serum oxytocinase (Fekete, 1930; Page, 1946; Werle & Semm, 1956), which by splitting the peptide linkage between the cysteine and tyrosine residues inactivates oxytocin (Tuppy & Nesvadba, 1957). Golubow, Chan & Du Vigneaud (1963) have shown recently that desamino[1]-oxytocin is not inactivated by the serum of pregnant women. The lack of the free amino group makes the molecule resistant to oxytocinase. The question arises whether the remarkably high activity of desamino[1]-oxytocin on the human uterus *in situ* is due to its resistance to oxytocinase. The fact that desamino[1]-oxytocin is also more potent than oxytocin if compared on the cat uterus *in situ* and on the chicken blood pressure, that is, in species which are known to have no serum oxytocinase, speaks strongly against such a hypothesis. Furthermore our tocographic studies do not suggest a different duration of action nor do they show any qualitative differences between desamino[1]-oxytocin and oxytocin. The tocographically recorded effects of equipotent doses of both compounds cannot be distinguished.

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