effect relationships. Vincristin is about 10fold more active than vinblastin (see also the table), but the slope is similar, which would suggest a similar mechanism of action. On the contrary, duration and shape of the action potential remain unaltered, which probably means that the differential permeability to ions is not modified. The threshold for excitability increases proportionally to the concentration of the drugs, the vincristin being about 10fold more powerful than vinblastin (see table). Repetitive firing is slightly decreased, possibly in connection with the increase of the threshold. The membrane potential is not modified.

Isoactive concentrations

Drug	25% decrease of the amplitude of the AP	25% increase of the threshold
Vinblastin	8.2 ± 0.73	7.2 ± 0.68
Vincristin	0.9 ± 0.08	0.7 ± 0.04

Mean ± S. E.; µg/ml; n = 6

In conclusion, the parameters which are more clearly modified by both alkaloids, are the amplitude of the action potential and the threshold for excitability. The effects I have described are brought about by the drugs within a few seconds from the beginning of the perfusion and are promptly reversible upon withdrawal. Presumably, these early alterations of the electric parameters are not correlated to the morphological alterations described, which are likely to be a slower and not so promptly reversible effect of the drugs.

The effects of both vincristin and vinblastin have been found to be almost completely antagonized by acetylcholine (figure 2). The antagonism is not unspecific, since acetylcholine does not prevent comparable reductions of the action potential brought about by the local anesthetic procaine under the same experimental conditions. A vinblastin-acetylcholine interaction has been described at the postsynaptic membrane (in the superior cervical ganglion of the cat), where it has been shown that vinblastin has an antiacetylcholine action[6].

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[2-o-Iodotyrosine]-oxytocin and [2-o-methyltyrosine]-oxytocin: Basic pharmacology and comments on their potential use in binding studies[1]

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Summary. Both [2-o-iodotyrosine]-oxytocin and [2-o-methyltyrosine]-oxytocin display only weak vasopressor and antidiuretic effects on rats. They inhibit the in vitro uterotonic action of oxytocin; this inhibition is not fully competitive. It is concluded that they are not suitable as markers for studies of uterine receptor for oxytocin.

Owing to the relative ease of preparation and the high specific radioactivities which can be achieved, peptide hormones with radioactive iodine have been extensively used in radioimmunoassay and are increasingly employed for the study of interactions with cellular binding sites, in particular the 'receptor' sites at which they initiate their biological responses. The iodinated analogues of oxytocin[3-5] and arginine vasopressin[6,7] have been considered for such studies several times. Whereas their use in a radioimmunoassay does not seem to create any particular problems, their relevance for receptor studies might be rather restricted for 2 reasons. First, a considerable binding to 'nonreceptor' sites (denoted – not quite correctly – as 'nonspecific' binding by some authors) does not allow one to follow the hormone-receptor interactions themselves, whether in a 'direct' or in a displacement experiment. This was indeed shown for the binding of iodinated arginine vasopressin to the plasma membranes of renal medulla[7]. Second, it is obviously necessary that the iodinated hormone binds to the receptor sites with a sufficiently high affinity. The example of renal medulla mentioned above[7] demonstrates that the binding of iodinated (or, more generally, 3-substituted tyrosine) analogues of neurohypophyseal hormones to their receptors might be rather weak. The strength and the mode of binding of iodinated oxytocin to the uterus receptor is not known so far and not easy to investigate directly. Therefore, we employed in this study a pharmacological approach based on the antagonistic properties of [Tyr(3-I)[2]]-oxytocin (abbreviation: IOT) towards oxytocin on the isolated rat uterus. In order to demonstrate the steric effect of substitution at the 3-position in the aromatic ring of tyrosine upon this binding, we have further studied the stereoisomeric 3-methylated analogue, [Tyr(3-Me)[2]]-oxytocin (abbreviation: MOT) in the same type of experiment. The calculated pA_2-values[8] – by definition, logarithms of hormone-receptor association constants – can then be taken as a measure of the affinity of these analogues to their receptors. Some additional pharmacological characteristics of the 2 substances have also been investigated and are mentioned in this paper.

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Experimental. IOT and MOT were synthetized by conventional procedure and purified by Sephadex chromatography (P. Marbach, D. Jarvis and J. Rudinger, in preparation). The synthetic oxytocin (OT) and lysine vasopressin (LVP) used as standards were synthetized by Ferring AB, Malmö (Sweden); the former substance was purified by counter-current distribution in n-butanol-acidic acid-water, the latter on a CM-Sephadex column as described earlier[9].

The uterotonic activity was assayed on an isolated isometric uterus taken from rats in natural estrus and suspended in van Dyke-Hastings medium according to Munsick[10] with or without magnesium. The four-point assay and the cumulative dose method[11] were employed. For inhibition studies, cumulative curves for OT in the presence of 10^{-7} to 3.5×10^{-6} M IOT or 10^{-6} to 5×10^{-6} M MOT were recorded and pA_2-values calculated – whenever possible – as indicated in the literature[11].

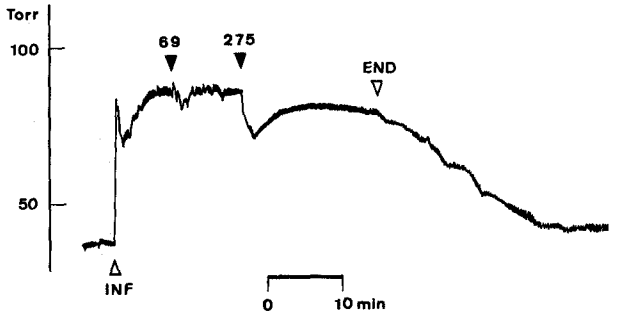

Fig. 1. Inhibition by IOT of LVP effect on rat blood pressure. IOT injections at symbols ▼; doses in nmoles indicated. Male rat 393 g. Infusion rate of LVP 0.6 nmoles/h. Ordinate: mean arterial blood pressure.

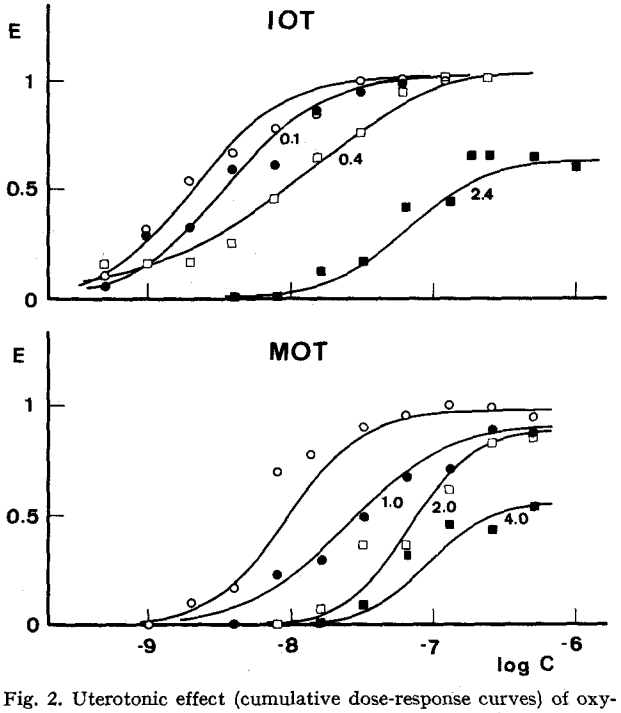

Fig. 2. Uterotonic effect (cumulative dose-response curves) of oxytocin in the absence (open circles) and presence of IOT and MOT (concentration indicated for each curve). Lines are computed fits (see text). E, isometric contraction given as fraction of the maximal attainable contraction with oxytocin in the absence of antagonists. C, concentration of oxytocin in organ bath (M).

Male rats in urethane anesthesia premedicated with Dibenzyline were used for the measurements of vasopressor effects[12]. The mean arterial blood pressure was recorded by means of a Statham pressure transducer in the left carotid artery. The substances were administered i. v. in the left external jugular vein. In inhibition experiments, either IOT was administered together with LVP and the vasopressor effect was compared with the same dose of LVP alone, or alternatively, LVP was applied as a permanent infusion (0.6–2.4 nmoles/h) and the influence of IOT on the steady state vasopressor effect was studied. The antidiuretic activity was studied as described earlier[13], using both OT and LVP as standards. The same procedures as described above were used for investigations of inhibition properties.

Results. The antidiuretic effects of both IOT and MOT were very low, both exhibiting about 0.01 IU/μmole (i.e., about 3% of antidiuretic potency of OT). No inhibitory effects were observed. There were no blood pressure effects observable up to a dose of 0.28 μmoles for IOT and 0.26 μmoles for MOT. The former substance, however, showed a slightly inhibitory effect on the LVP administered by permanent infusion (1.2 nmoles/h, figure 1) in some rats. These effects were not entirely reversible. Also, no uterotonic activity of the 2 substances in the presence or in the absence of magnesium could be demonstrated.

In the absence of Mg^{2+}, both substances inhibit the effect of oxytocin on the rat uterus (cumulative – dose procedure). At rather low concentrations of IOT and MOT (10^{-7} to 5×10^{-7} M), the inhibition seems to be of a competitive nature, pA_2 values of 7.05 ± 0.08 (6) for IOT and 6.79 ± 0.15(3) for MOT being obtained (arithmetic means ± SE; in parentheses: number of experiments). However, higher concentrations lead to a clearly non-competitive antagonistic effect characterized by a decrease in the maximal contraction and by a shift of the dose-response curve to the right along the dose axis. With increasing inhibitor concentration the log dose-response curves are gradually shifted to the right and, starting at a more or less sharply defined inhibitor concentration, a visible decrease of the maximal effect follows. This is visualised in figure 2, where the experimental points are fitted by a 'sigmoidal' dose-effect function[14,15]

$$E = E_{max}/([C_{0.5}/C]^h + 1)$$

which describes a relationship between an effect E and a concentration C (E_{max} being the maximal effect, $C_{0.5}$ the concentration inducing the half-maximal effect $E_{max}/2$, h a power coefficient, the nature of which is not precisely known). The computer analysis of the data was carried out with a program described earlier[16]; curves in figure 2 represent a computed nonlinear least square fit. The h-coefficients were approx. 1.2–2.1 and there was no convincing relation found between their values and the inhibitor concentration, although some increase of their values with increasing inhibitor concentration was registered. After washing out the inhibitor, the uterus recovered within 1–3 h, to give a full response to oxytocin.

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All these effects are qualitatively as well as quantitatively similar for both substances. We therefore conclude that they can be accounted for by the steric effect of the ortho-substituents rather than by changes in the dissociation of the hydroxyl group of tyrosine induced by a substituent located ortho to it.

Discussion. Figure 2 documents quite clearly that the inhibition of oxytocin effect on the rat uterus by IOT and MOT is not purely competitive. Over a broad range of inhibitor concentrations the pattern of the dose-response curves is very similar to that found for irreversible blockade of receptors in the case of so-called receptor reserve[17]. From a chemical point of view, it seems very unlikely that the substituent groups can provide a strong link to the receptors such as to cause an irreversible decrease in their number. On the other hand, however, the irreversibility of inhibitor binding is only a relative phenomenon, indicating that the 'active' substance is more readily released from the binding site that the inhibitor itself. In the series OT–MOT–IOT, such a difference in the disappearance rate from the receptor can be accounted for by increasing lipophilicity. It is instructive to mention in passing that prostaglandins were also found to inhibit the hydroosmotic effect of an oxytocin analogue, [Tyr(Me)^2]-oxytocin, showing the 'receptor-reserve'-pattern[18]. This, in our opinion, is a reflection of the great difference in lipophilicity between the 2 agents which determines their tightness of binding to the receptor and/or their effective concentration in the vicinity of the receptor. Such factors may also be operative in cellular or subcellular (e.g., membrane) preparations.

We are aware that any attempt to generalize would be pure guesswork. At any rate, however, the empirical evidence itself suggests that IOT is not very suitable for displacement experiments in uterus receptor studies and the interpretation of any such investigations in which iodotyrosine analogues are employed as markers deserves great caution.

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Potentiation by crude kallikrein of the myotropic effect of angiotensin I in the isolated rabbit aortic strip

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Summary. Crude kallikrein (Padutin®), but not pure kallikrein, when preincubated with angiotensin I caused a potentiation of the myotropic effect of decapeptide on the isolated continuously superfused rabbit aortic strip. Addition of converting enzyme inhibitor, SQ 20881, to the medium inhibited this potentiation. The potentiation by crude kallikrein of the myotropic effect of angiotensin I is probably due to the conversion of decapeptide to octapeptide angiotensin II. This study indicates that Padutin is not a pure kallikrein preparation and probably contains a kininase fraction which causes the conversion of angiotensin I.

The conversion of angiotensin I (A I) to angiotensin II (A II) has been shown to be mediated by an enzyme present in many tissues especially in the lung and mesenteric circulation [2–4]. On the other hand, it has been shown that converting enzyme also causes the degradation of bradykinin and the synthetic inhibitor of this enzyme (nonapeptide, SQ 20881)[5] produces an inhibition in the conversion of A I but potentiates the effects of bradykinin[6]. The data presented in this paper indicate that crude kallikrein (Padutin®), but not pure kallikrein, when preincubated with A I caused a potentiation of the myotropic effect of A I on the continuously superfused rabbit aortic strip.

Material and method. The myotropic activity of A I was determined on the continuously superfused spirally cut rabbit aortic strips[7] as described previously[8]. This

Potentiation by crude kallikrein (Padutin®) of the myotropic effect of A I on the isolated continuously superfused rabbit aortic strips

Concentrations of angiotensins (ng/ml)		Control responses to angiotensins	Responses to A I incubated with Padutin (0.1 U/ml) Without SQ 20881	With SQ 20881 (50 ng/ml)
A I	5	0	23.0 ± 2.4	8.6 ± 0.9
	10	0	37.3 ± 2.8	16.0 ± 1.6
	20	0	66.1 ± 3.1	31.7 ± 1.1
	40	3.2 ± 1.0	85.4 ± 2.5	43.2 ± 2.1
	80	8.0 ± 1.1	–	65.5 ± 2.0
A II	5	28.7 ± 2.5	–	–
	10	40.0 ± 2.8	–	–
	20	76.6 ± 3.3	–	–
	40	96.6 ± 5.5	–	–

Per cent of maximum responses measured on the recorder (mean ± SEM of 10 experiments).

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