neuromembrane in the chloride-free condition, in the same manner as in the physiological medium. We confirmed that β-hydroxy glutamic acid (BHGA, erythro-L-type) also inhibited the PON in the chloride-free condition. We conclude that the inhibitory effect of these substances on the PON is probably not due to the membrane permeability increase to chloride ions.

Walker et al.[14] reported that L-glutamic acid and ibotenic acid show the same effect (inhibitory or excitatory) on identifiable giant neurones of a European garden snail (Helix aspersa). They suggested that the 2 amino acids will act on the same receptor of the neuromembrane under the same ionic mechanism (their inhibitory effect is due to the membrane permeability increase to both chloride and potassium). Concerning the PON of Achatina fulica Férussac, not L-glutamic acid, but erythro-β-hydroxy-L-glutamic acid and 2 heterocyclic amino acids (ibotenic acid and quisqualic acid) show the same effect. We suggest the possibility that the 3 substances act on the same sites of neuromembrane under the same ionic mechanism. Unlike the results of Walker et al. using Helix aspersa, the 3 amino acids are not considered to increase the PON membrane permeability to chloride.

Lea and Usherwood[15,16] suggested that the conductance increase of the locust (Schistocerca gregaria) muscle fibre membrane caused by ibotenic acid is due to the membrane permeability increase of chloride. Their findings on the effect of ibotenic acid would be different from the case of the PON neuromembrane.

14 R. J. Walker, G. N. Woodruff and G. A. Kerkut, Comp. Gen. Pharmac. *2*, 168–174 (1971).
15 T. J. Lea and P. N. R. Usherwood, Comp. Gen. Pharmac. *4*, 333–350 (1973).
16 T. J. Lea and P. N. R. Usherwood, Comp. Gen. Pharmac. *4*, 351–363 (1973).

The effect of vinblastin and vincristin on single nerve fibres

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Summary. The perfusion of the node of Ranvier with vinblastin or vincristin reduces the amplitude of the action potential within a few seconds. Vincristins is 10fold more active than vinblastin. Upon withdrawal, the effect is promptly reversible and it is antagonized by acetylcholine.

Vinblastin and vincristin, the 2 main alkaloids of Vinca rosea, are endowed with a marked neurotoxicity even at current therapeutical doses[1]. It has been maintained that the primary alteration is an impairment to the axonal transport through degenerative changes in nervous tissue[2]. The propagation of the action potentials would be altered only secondarily, some time after the axoplasmic transport has completely stopped[3].

I have investigated, in the isolated nerve fibre of the untreated frog, the modifications of the action potentials following perfusion of the node of Ranvier with solutions containing vinblastin or vincristin; in this paper much faster effects are reported of both alkaloids on the properties of the membrane of the nerve fibre.

Method. I have studied the action of vinblastin and vincristin on the following parameters of the electric activity in isolated nerve fibres: amplitude and duration of the action potential, threshold for electrical excitability and membrane potential. Furthermore I searched for modifications of the repetitive firing which may be elicited in sensory fibres by a maintained electric stimulus[4] (about 30 msec). Single myelinated nerve fibres were isolated from the frog's sciatic nerve and transferred to a special chamber[5] where one node of Ranvier was continuously superfused. One of the neighbouring nodes was stimulated and the electric activity of the perfused node was recorded according to the air-gap technique[5]. The liquid of perfusion was a balanced salt solution for amphibia (110.5 NaCl, 2.5 KCl, 1.8 $CaCl_2$ and 2.4 $NaHCO_3$, all in mM/l) in which either vincristin sulphate or vinblastin sulphate (Eli Lilly & Company, Indianapolis, USA) could be dissolved. The fibres were stimulated by rectangular pulses of 0.2 msec with the frequency of 1 per sec.

Results. The amplitude of the action potential was found to be reduced by both drugs. Figure 1 shows the dose-

1 E. B. Casey, A. M. Jellife, Pamela M. Lequesne and Yvonne L. Millett, Brain *96*, 69 (1973).
2 M. L. Shelanski and H. Wisniewski, Arch. Neurol. Chicago *20*, 199 (1969).
3 W. O. McClure, Adv. Pharmac. Chem. *10*, 185 (1972).
4 H. Schmidt and R. Staempfli, Helv. Physiol. Acta *22*, C 143 (1964).
5 R. Staempfli, Ann. N. Y. Acad. Sci. *81*, 265 (1959).

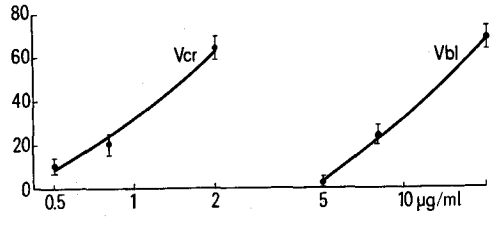

Fig. 1. Per cent decrease of the amplitude of the action potential (ordinate) at different concentrations (abscissa) of Vinblastin (Vbl) and Vincristin (Vcr).

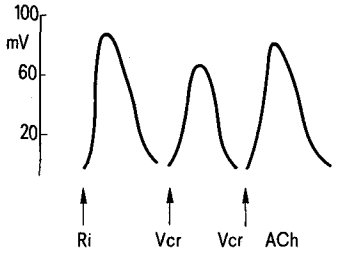

Fig. 2. Action potentials with simple Ringer (Ri), Vincristin (1µg/ml) (Vcr) and Vincristin + Acetylcholine (1 µg/ml + 1 mg/ml) (Vcr + ACh).

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].

6 J. M. Trifaro, B. Collier, A. Lastowecka and D. Stern, Molec. Pharmac. N. Y. *8*, 266 (1972).

[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.

1 Supported by the Swiss National Science Foundation, grant No. 3.2080.73.

2 Deceased 30 April 1975.

3 E. E. Thompson, P. Freychet and J. Roth, Endocrinology *91*, 1199 (1972).

4 P. Marbach and J. Rudinger, Experientia *30*, 696 (1974).

5 G. Flouret, S. Terada, T. Nakahara, S. H. Nakagawa and O. Hechter, in: Peptides: Chemistry, Structure and Biology, p. 751. Ed. R. Walter and J. Meienhofer. Ann Arbor Science Publishers Inc., Ann Arbor, Mich. 1975.

6 H. Wagner and V. Maier, Biochem. Soc. Trans. *3*, 1025 (1975).

7 R. E. Weitzman and D. A. Fischer, Communication at the V. Int. Congress of Endocrinology, Hamburg 1976 (Abstract No. 185).

8 E. J. Ariëns, A. M. Simonis and J. M. van Rossum, in: Molecular Pharmacology, vol. I, p. 119. Ed. E. J. Ariëns. Academic Press, New York, N.Y. 1964.