PREMIER MINISTRE

COMMISSARIAT A
L'ÉNERGIE ATOMIQUE

Pharmacological characterization of the oxytocic peptides in the pituitary of a marine teleost fish (Pollachius virens)

par

H. HELLER and B.T. PICKERING
The University, Bristol
and
J. MAETZ and F. MOREL
Commissariat à l'Energie Atomique

Rapport CEA n° **2062**

1961

CENTRE D'ETUDES
NUCLÉAIRES DE SACLAY

CEA 2062 - HELLER H., PICKERING B. T., MAETZ J., MOREL F.

Caractérisation pharmacologique des peptides ocytociques dans la glande pituitaire d'un poisson marin teleost (pollachius virens) (1961).

Sommaire. — Mise en évidence dans la neurohypophyse d'un poisson marin (pollachius virens) d'un peptide à activité ocytocique différent de l'ocytocine des mammifères et de l'arginine vasotocine.

CEA 2062 - HELLER H., PICKERING B. T., MAETZ J., MOREL F.

Pharmacological characterization of the oxytocic peptides in the pituitary of a marine teleost fish (pollachius virens) (1961).

Summary. — Demonstration of the existence, in the neurohypophyse of a marine fish (pollachins virens), of an oxytocic-active peptide different to the oxytocine in mammals and to the arginine vasotocine.

(*Reprinted from Nature, Vol. 191, No. 4789, pp. 670-671, August 12, 1961*)

PHARMACOLOGICAL CHARACTERIZATION OF THE OXYTOCIC PEPTIDES IN THE PITUITARY OF A MARINE TELEOST FISH (*POLLACHIUS VIRENS*)

By Prof. H. HELLER and B. T. PICKERING
Department of Pharmacology, The University, Bristol
AND
J. MAETZ and F. MOREL
Departement de Biologie, Commissariat a l'énergie atomique, Saclay, Gif-sur-Yvette, Seine et Oise, France

PITUITARY extracts from teleost fishes have been shown to contain at least two oxytocic substances[1,2]. In the pollock or pollack (*Pollachius virens*) one of these substances has been identified as arginine^8–oxytocin (arginine vasotocin) by amino-acid analysis[2,3]. Arginine^8–oxytocin has also been chemically characterized in neurohypophysial extracts from frog[4] and chicken[5]. Chromatographic[2] and pharmacological[2,6] evidence has been advanced for the presence of arginine^8–oxytocin in the neurohypophysis of reptiles as well as in that of other amphibians and birds.

The second oxytocic principle found in pollock pituitary extracts was found to have many of the properties of oxytocin. Relative to its oxytocic potency, it showed very weak antidiuretic activity and, like oxytocin, had a much lower frog water balance activity than vasotocin[1]. When chromatographed on paper in butanol / acetic acid / water (4 : 1 : 5) (refs. 1 and 2) or eluted from a column of 'Amberlite *CG*-50' (ref. 2) it was found in the same position as oxytocin.

The 'second' oxytocic peptide in pollock pituitary extracts has now been compared with oxytocin ('Syntocinon', Sandoz) by further pharmacological tests. The sample of peptide used was prepared by paper chromatography of the material obtained from the region of oxytocin when pollock pituitary extract was eluted from a column of 'Amberlite *CG*-50' as in ref. 2. The active eluate from the paper chromatogram was freeze-dried and the resulting solid dissolved in 0·25 per cent acetic acid containing 0·5 per cent

chlorbutol as preservative. This solution was used in all the biological assays described here.

The following tests were performed (the same batch of 'Syntocinon' = synthetic oxytocin, was used as standard throughout): (*a*) *Isolated rat uterus assay with and without magnesium:* Munsick[7] has shown that the action of many synthetic analogues of oxytocin on the isolated rat uterus is potentiated by the presence of magnesium in the suspension fluid. He showed also that the degree of potentiation varied from one peptide to the other. Accordingly, the unidentified pollock peptide was assayed on rat uteri suspended in either Munsick's[7] modified van Dyke–Hastings solution[8] without magnesium ions or in the same solution containing 0·5 m*M* Mg^{++}/l. The solution of the pollock peptide had a potency of 7 *U*/ml. in the absence of magnesium, but in the presence of this ion its potency was increased to 28 *U*/ml. Since 'Syntocinon' was the reference standard in both sets of assays, the increase in potency of the pollock peptide clearly differentiates it from oxytocin. (*b*) *Avian depressor assay*[9] *:* Estimations of the potency of the pollock peptide by this method gave a mean of 16·3 *U*/ml. compared with 7 *U*/ml. in assays on the isolated rat uterus without magnesium. (*c*) *Effect on sodium transport through the isolated frog skin* (natriferic activity[10]): unlike oxytocin, the unknown pollock peptide almost completely lacks the power to stimulate the sodium current produced by *Rana esculenta* skin *in vitro.* The mean natriferic potency was found to be 350 m*U*/ml. or 1/20 of the oxytocic activity. (*d*) *Effect on water movement through isolated frog bladder* (gravimetric assay[11,12]): as compared with oxytocin, the second pollock principle exhibited only a slight enhancing effect on the osmotic permeability of the *Rana esculenta* bladder. The mean activity was 750 m*U*/ml. or 1/9 of the oxytocic activity. (*e*) *Effect on water movement through the isolated frog skin* (volumetric assay[13]): The potency of the unidentified pollock peptide to enhance the net water flux through the isolated skin of *Rana esculenta* was very low, that is, about 350 m*U*/ml. or 1/20 of the oxytocic activity. (*f*) *Effect on water uptake by intact frogs:* in one *in vivo* experiment (21 animals) on *Rana esculenta,* the water-balance activity of the solution containing the unidentified pollock peptide was found to be less than 1 *U*/ml., that is, its potency in this test was less than 1/7 of that in assays on the rat uterus without magnesium ions. Low sensitivity of 'winter frogs' prevented further experiments.

The low potency of the unknown 'second' pollock oxytocic principle in all the amphibian tests is in

striking contrast to the high potency shown by the 'first' oxytocic principle which has been chemically characterized as arginine^8–oxytocin[2,3]. Compared with oxytocin, for example, the latter was found in a few preliminary experiments to be about 20 times more potent on the sodium transport across frog skin and 200 times more active on the water movement through the isolated frog bladder. It was already known to be about fifty times more active in the frog water balance test *in vivo*[2].

Fig. 1 shows the difference in the potencies of the two oxytocic principles found in pollock pituitary extracts with reference to their action on sodium transport across frog skin.

The effect of oxytocin on the renal water excretion of rats varies according to the conditions of the experiment[14,15], but it has been shown that the hormone has a weak antidiuretic action in intact rats with a high water load. Assayed against 'Pitressin', 'Syntocinon' was found to have an antidiuretic activity corresponding to 1·1 per cent of its oxytocic potency[15]. When the unidentified pollock peptide solution was compared with 'Pitressin', its antidiuretic action was equal to a mean of 13 m*U*/ml. or to 0·2 per cent of its oxytocic activity.

In a few preliminary antidiuretic assays[16] on *Rana esculenta* the antidiuretic activity of the unknown pollock peptide was likewise found to be very weak, while the 'first' oxytocic peptide showed a high antidiuretic activity similar to that of arginine^8– or lysine^8–oxytocin.

Table 1 shows the 'pharmacological spectrum' of the solution containing the unidentified oxytocic peptide in pollock pituitary extracts. The standard in each of the assays shown was synthetic oxytocin ('Synto-

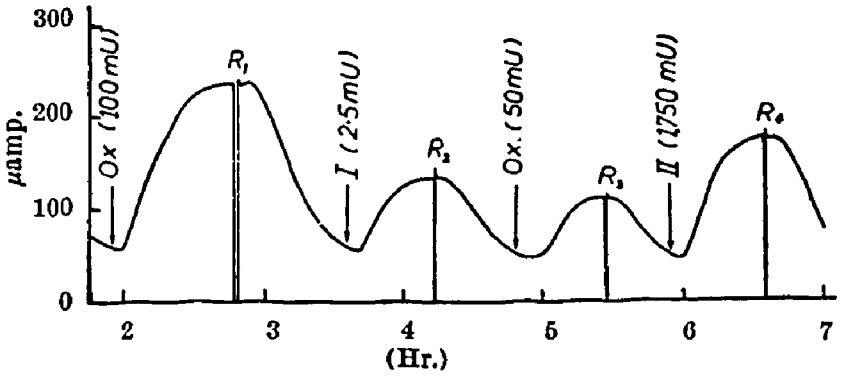

Fig. 1. Comparison of the effects of the two oxytocic pollock peptides and of oxytocin on the 'sodium current' (ref. 10) produced by frog skin. Ordinate: 'short circuited current' (in μamp.); abscissa: time, hr. Peptides added at arrows (in brackets: oxytocic = rat uterus activity). *Ox* = synthetic oxytocin; *R* = rinsing; I = the first oxytocic principle characterized as arginine^8–oxytocin. Note that it was about 20 times *more* potent than oxytocin. II = The second oxytocic principle. Note that it was about 20 times *less* potent than oxytocin

Table 1. POTENCY OF THE UNIDENTIFIED ('SECOND') OXYTOCIC POLLOCK PEPTIDE ASSAYED AGAINST OXYTOCIN ('SYNTOCINON', SANDOZ) (Note that, by definition, the activity ratios would be 1, had the unknown substance been oxytocin)

Assay method	Potency (*U*/ml.)	Ratio of activity to rat uterus ($-Mg^{++}$) potency
Rat uterus without Mg^{++}	7	1
Rat uterus with 0·5 m*M* Mg^{++}/l.	28	4
Chicken blood pressure	16·3	2·3
Natriferic activity (isolated frog skin)	0·35	0·05
Water-balance activity (isolated frog skin)	~0·35	~0·05
Water-balance activity (isolated frog bladder)	0·75	0·11
Water-balance activity (intact frog)	<1	<0·15

cinon'). If, therefore, the pollock peptide had been identical with oxytocin, the activity ratio (last column of the table) would have been unity in each type of assay. The fact that all the assays on amphibians gave activity ratios very much less than unity indicates that oxytocin, if present at all, could only have been present in very small amounts.

Until the unknown peptide is completely purified, it cannot be excluded that the preparation used in the present investigation contained other very similar peptides. However, the results presented are sufficient to demonstrate that teleost pituitary glands may contain not only arginine[8]-oxytocin (arginine vasotocin) but also another oxytocic peptide not found in the neurohypophysis of mammals. It would thus appear that the diversity of neurohypophysial active peptides in the vertebrate phylum is greater than hitherto assumed.

[1] Pickering, B. T., and Heller, H., *Nature*, **184**, 1463 (1959).
[2] Heller, H., and Pickering, B. T., *J. Physiol.*, **155**, 98 (1961).
[3] Heller, H., and Pickering, B. T., *J. Physiol.*, **152**, 56P (1960).
[4] Acher, R., Chauvet, J., Lenci, M.-T., Morel, F., and Maetz, J., *Biochim. Biophys. Acta*, **42**, 379 (1960).
[5] Chauvet, J., Lenci, M.-T., and Acher, R., *Biochim. Biophys. Acta*, **38**, 571 (1960).
[6] Sawyer, W. H., Munsick, R. A., and van Dyke, H. B., *Nature*, **184**, 1484 (1959).
[7] Munsick, R. A., *Endocrinology*, **66**, 451 (1960).
[8] van Dyke, H. B., and Hastings, A. B., *Amer. J. Physiol.*, **83**, 563 (1928).
[9] *British Pharmacopoeia* (The Pharmaceutical Press, London, 1958).
[10] Maetz, J., Morel, F., and Race, B., *Biochim. Biophys. Acta*, **36**, 317 (1959).
[11] Bentley, P. J., *J. Endocrin.*, **17**, 201 (1958).
[12] Sawyer, W. H., *Endocrinol.*, **66**, 112 (1960).
[13] Morel, F., Maetz, J., and Lucarain, Cl., *Biochim. Biophys. Acta*, **28**, 619 (1958).
[14] Brunner, H., Kuschinsky, G., and Peters, G., *Arch. Exp. Path. Pharmakol.*, **228**, 457 (1959).
[15] Berde, B., and Cerletti, A., *Helv. Physiol. Acta*, **14**, 129 (1956).
[16] Uranga, J., and Sawyer, W. H., *Amer. J. Physiol.*, **198**, 1287 (1960).

Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

FIN