Pharmacological properties of a cholinergic-receptor antiserum

F. BERTI, F. CLEMENTI*,
B. CONTI-TRONCONI & C. OMINI

Department of Pharmacology and Cytopharmacology Center of the C.N.R., School of Medicine, University of Milan, Italy and Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Milan, Italy

A cholinergic receptor-rich fraction was isolated from the electric organs of '*Torpedo marmorata*' by centrifugation on a sucrose density gradient, according to the method of Cohen, Weber, Huchet, Maud & Changeux (1972).

Rabbits treated subcutaneously with 1 mg/kg protein of the receptor-rich fraction emulsified in Freund's adjuvant, developed a progressive flaccid paralysis which affected the hing-legs first, and which led, at the end, to complete head drop due to relaxation of the neck muscles.

At variance with the data reported by Patrick & Lindstrom (1973), the animals did not show any respiratory distress.

Complete recovery from the diffuse paralysis could be obtained after intravenous injection of prostigmine.

Sera obtained from treated rabbits showed during the immunodiffusion test the presence of antibodies versus cholinergic receptors.

Pharmacological activity of these sera was tested in several preparations *in vivo* and *in vitro*. A clear antagonism against acetylcholine-induced contractions could be demonstrated using the rectus abdominis muscle of the frog.

In the 'phrenic nerve-diaphragm' preparation of the rat, the sera exerted a progressive and severe inhibition of the neuromuscular transmission, and this effect could be reversed by eserine.

Reference

COHEN, J.B., WEBER, M., HUCHET, MAUD & CHANGEUX, J.P. (1972). Purification from Torpedo marmorata electric tissue of membrane fragments particularly rich in cholinergic receptor protein. *FEBS Letters*, **26**, 43-47.

PATRICK, J. & LINDSTROM, H. (1973). Autoimmune response to acetylcholine receptor. *Science*, **180**, 871-872.

Preliminary observations on the effects of eledoisin on ruminant's forestomach and abomasum

C. BERETTA*, R. FAUSTINI, P. ORMAS & G. POMPA

Institute of Veterinary Pharmacology and Toxicology, University of Milan, Italy

The endecapeptide eledoisin is well known for its powerful effects on vascular and extravascular smooth muscles (Anastasi & Erspamer, 1962; Erspamer & Falconieri Erspamer, 1962; Erspamer & Glaesse, 1963).

Contrary to what occurs in other species where the peptide affects blood pressure either with hypotensive or biphasic effects (Erspamer & Glaesser. 1963; Miele & De Natale, 1967), in sheep it lacks cardiovascular actions eliciting only inconstant and moderate hypertension (due to catecholamines release) at very high dosages (0.5-3.0 μg/kg i.v.) (Ormas, Pompa, Beretta & Faustini, 1974).

We have now tested synthetic eledoisin administered by rapid i.v. injection to check its activity on the smooth musculature of forestomach of sheep in view of a possible therapeutic use in forestomach atonia or hypomotility.

Reticular, omasal, ruminal (dorsal sac) and abomasal movements in ten sheep (Italian breed) weighing 45-55 kg and fasting from 24 h under sodium pentobarbitone (25-30 mg/kg i.v.) anaesthesia were recorded by means of strain-gauges connected to carrier-preamplifiers of an ink-pen polygraph (OTE mod. R 35 g-t).

The results obtained can be summarized as follows:

(1) Eledoisin stimulated the motility both of three forestomachs and of the abomasum.

(2) The threshold doses were 25-50 ng/kg for omasal musculature and 50-100 ng/kg for that of the other stomachs.

(3) The recorded effects proved to be proportional with the doses administered both for intensity and duration except in the reticulum where doses higher than 0.5 μg/kg provoked initially more frequent and smaller contractions.

(4) As regards reticulum, eledoisin was able to excite the rythmical activity both when this was absent or blocked by a previous administration of caerulein (10-25 ng/kg i.v.).

(5) The pretreatment of the animals with atropine sulphate (1.5 mg/kg i.v.), mepyramine HCl (2 mg/kg i.v.), hexamethonium HBr

(8.5 mg/kg i.v.), phentolamine metansulphonate (5 mg/kg i.v.), dihydroergotamine tartrate (0.2 mg/kg i.v.), propranolol HCl (3.0 mg/kg i.v.) and BOL (0.25 mg/kg i.v.) never affected the stimulating properties of eledoisin.

(6) Eledoisin did not have a significant effect on blood pressure at doses tested.

(7) Synthetic substance P (Beckman) proved to be inactive in four out of the five sheep to which it was injected for comparison despite the high doses tested (1-1.5 μg/kg i.v.).

The results obtained suggest that sheep may be a good experimental model to study the stimulating properties of eledoisin on extravascular smooth musculature without cardiovascular interferences and moreover that this peptide might be a new therapeutic tool for the treatment of forestomach atonia in ruminants.

References

ANASTASI, A. & ERSPAMER, V. (1962). Occurrence and some properties of eledoisin in extracts of posterior salivary glands of Eledone. *Br. J. Pharmac.*, 19, 326-336.

ERSPAMER, V. & FALCONIERI ERSPAMER, G. (1962). Pharmacological actions of eledoisin on extravascular smooth muscle. *Br. J. Pharmac.*, 20, 337-354.

ERSPAMER, V. & GLAESSER, A. (1963). The action of eledoisin on the systemic arterial blood pressure of some experimental animals. *Br. J. Pharmac.*, 20, 516-527.

MIELE, E. & DE NATALE, G. (1967). Modification of pressor effects of some vasoactive polypeptides in the rat by guanethidine, propranolol and related agents. *Br. J. Pharmac.*, 29, 8-15.

ORMAS, P., POMPA, G., BERETTA, C. & FAUSTINI, R. (1974). The effects of some peptides on the systemic blood pressure of sheep. (in press).

Biological characterization of some cyclopentane analogues of muscarone and muscarine

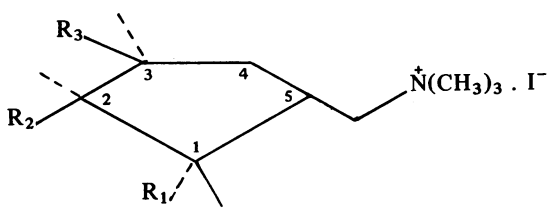
M. CINGOLANI, G. GAMBA, P. PIGINI, L. RE & L. ROSSINI*

Laboratory of Pharmacology, Institute of Experimental and Clinical Medicine, University of Ancona Medical School, 60100, Italy

M. GIANNELLA, F. GUALTIERI, C. MELCHIORRE & M. PIGINI

Institute of Organic and Pharmaceutical Chemistry, University of Camerino, 62032, Italy

We have recently synthetized the following derivatives of muscarone ('A'), and muscarine ('E') (as a mixture of geometric and optical isomers if not otherwise indicated geometric isomerism is indicated with reference to C5 side chain):



A: 1 = 0; R_2 = H, CH_3; R_3 = 0;
B: R_1 = H, H; R_2 = H, H; R_3 = 0;
C: R_1 = H, H; R_2 = H, CH_3; R_3 = 0;
D: R_1 = H, CH_3; R_2 = H, CH_3; R_3 = 0;
E: 1 = 0; R_2 = H, CH_3 (*cis*); R_3 = H, OH (*trans*);
F: R_1 = H, H; R_2 = H, H; R_3 = H, OH (*cis*);
G: R_1 = H, H; R_2 = H, H; R_3 = H, OH (*trans*);
H: R_1 = H, H; R_2 = H, CH_3 (*cis*); R_3 = H, OH (*cis*);
I: R_1 = H, H; R_2 = H, CH_3 (*trans*); R_3 = H, OH (*cis*);
L: 2-3 unsaturated; R_1 = H, H; R_2 = R_3 = H;
M: R_1 = H, H; R_2 = H, CH_3; R_3 = 0; 4 = CH_3; 5 = CH_2 (open ring);
N: R_1 = H, H; R_2 = H, CH_3 (*cis*); R_3 = H, OH (*trans*).

Compound 'C' has already been studied by us (Cingolani, Giannella, Gualtieri, Melchiorre, Pigini & Rossini, 1973a; Gualtieri, Giannella, Melchiorre & Pigini, 1974), and compound 'N' by Sundelin, Wiley, Givens & Rademacher (1973). Both compounds were more active than acetylcholine (Ach) when assayed on guinea-pig ileum, results implying that the electronic contribution of the ether oxygen of the furan ring, and/or the ester oxygen of Ach were not necessary for optimum receptor interaction (cf. Cingolani, Giannella, Gualtieri, Melchiorre, Pigini & Rossini, 1973b; Michelson & Zeimal, 1973). A more complete pharmacological analysis of a series of the isosteres may contribute to the characterization of a new series of Ach receptors, and allow for the revision of current hypotheses of cholinergic activation and control. Receptor purification appears feasible through affinity chromatography of matrix-bound derivatives with adequately long aliphatic arm-substituents of the new carbocyclic methylene analogue.