(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.

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Biological characterization of some cyclopentane analogues of muscarone and muscarine

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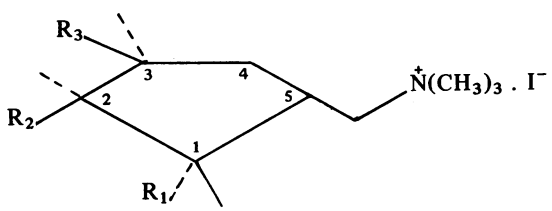
We have recently synthetized the following derivatives of muscarone ('A'), and muscarine ('E') (as a mixture of geometric and optical isomers if not otherwise indicated geometric isomerism is indicated with reference to C5 side chain):



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.

Parallel line assays according to Edinburgh procedures (1968, 1970) were performed with Ach bromide and/or chloride as the standard. Potency ratios (relative to Ach) were calculated from the ED_{50}s, obtained through the regression of the log concentrations of the compounds vs the angular transformate of the fractional effects. Statistical significance ($P \leqslant 0.01$) of the values of positions and slopes of the straight lines, and of their parallelism, was checked. The standard errors of mean values of the ED_{50}s were less than 10%.

Preliminary results were obtained on the BP of pithed rats (Wistar, Morini breeding, both sexes, 200-300 g), where the compounds with $R_3 = 0$ showed a characteristic profile of potency, always more than or equipotent to Ach for the hypotensive effect, followed by a hypertensive peak. In the atropinized animal (atropine sulphate, H_2O; i.v., 800 μg/kg) hypertension was greater, and disappeared after hexamethonium (chloride; i.v., 4 mg/kg). Nicotinic effects were also assayed on frog rectus abdominis (frog–Ringer, room temperature), and these potency ratios were obtained: 0.35 ('C'); 0.1 ('F', 'I'); 0.03 ('B', 'M'); 0.01 ('D'), and <0.01 ('G', 'H'). Compound 'D' increased the strength of the contractions of the rat-phrenic nerve diaphragm preparation (Krebs solution, 38° C) supramaximally stimulated at 10 and 50 Hz, and was able to counteract the effect of succinylcholine (chloride Midarine Wellcome; 2 μg/ml); all other compounds showed blocking action, greater at the higher frequency of stimulation, 50 to 100 times more powerful for 'C'. Their cholinergic depolarization was increased by succinylcholine, and/or Prostigmine (Roche; 10 μg/ml), and they produced spastic paralysis in the chick.

Muscarinic effects were assayed *in vitro* on rat jejunum, guinea-pig (Morini, both sexes, 200-300 g) ileum, ductus deferens, seminal vesicle (Tyrode, 38° C), tracheal chain (Krebs, 38° C), and isolated auricle (Ringer-Locke, 31° C), and frog heart (on Straub's cannula; frog Ringer, room temperature). One 'horizontal profile', that is the potency ratios for compound 'C' and for these last seven receptors, was: 2.4; 2.2; 0.65; 0.01; 120; 1.1; and 0.7 respectively, and the effects variously modified by atropine and hexamethonium. The intestinal contractions were slow and prolonged, an activity which may be related to the inhibition of acetylcholinesterase ($K_i \cong 1$ mM; measured according to Ellman, Courtney, Andres & Featherstone, 1961). Compound 'C' showed $\alpha = 0.45$ in seminal vesicle, whereas with all other preparations a unit intrinsic activity had been found. LD_{50} (i.p.; mice; 24 h observation) was equal to 8.07 mg/kg for the same compound. Examples of 'vertical profiles', that is the pattern of the potency ratios for two other preparations are: g.p. ileum: 2.2 ('C'); 0.02 ('B'); 0.01 ('I', 'L'); <0.01 ('D', 'F', 'G', 'H', 'M'), and tracheal chain: 120 ('C'); 0.5 ('F'); 0.3 ('D'); 0.2 ('B'); 0.1 ('L'); 0.05 ('M'); <0.01 ('H', 'G', 'I').

The singularities of the 'horizontal' vs the 'vertical' profiles prompted us to identify distinctive areas, that is definite patterns of structure-activity relationships, which characterize some new iso-receptors, a conclusion reached both for the nicotinic and the muscarinic effects.

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