Some pharmacological actions of alytesin and bombesin

Alytesin(I) and bombesin(II) are two natural tetradecapeptides recently isolated from methanol extracts of the skin of three European amphibians belonging to the family Discoglossidae. Alytesin has been found in the skin of *Alytes obstetricans*, bombesin in the skin of *Bombina bombina* and *Bombina variegata variegata*. Bombesin or a bombesin-like peptide is present also in the skin of *Bombina variegata pachypus* (Anastasi, Erspamer & Bucci, 1970).

(I) Pyr-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

(II) Pyr-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

The two polypeptides differ only in two amino-acid residues, the second and the sixth from the *N*-terminus, which are glycine and threonine in alytesin and glutamine and asparagine in bombesin.

Bombesin and alytesin can be easily demonstrated on paper chromatograms or electropherograms of crude or, much better, semi-purified extracts by means of colour reactions: Pauly reaction (histidine), *p*-dimethylaminobenzaldehyde reaction (tryptophan), and Sakaguchi reaction (arginine). Alytesin and bombesin may be accompanied by other compounds giving similar colour reactions.

The spectrum of biological activity of alytesin and bombesin is characteristic, and the distinction of this polypeptide family from other families is quite easy by means of parallel bioassay. The pharmacological study of bombesin and alytesin is in progress. So far, the following effects may be considered as well established for alytesin.

There was a hypertensive action in the dog, with marked tachyphylaxis. The threshold dose was less than 1 μg/kg by rapid intravenous infusion but the effect declined on continued administration of the polypeptide. The effect on blood pressure remained unchanged after pretreatment with α-adrenergic blocking agents. There was no cross-tachyphylaxis with Val^5-angiotensin. Alytesin was approximately ten times less potent than Val^5-angiotensin in its intensity of action, but hypertension elicited by alytesin lasted much longer.

It had potent stimulant action on the rat oestrous uterus being usually 2–4 times more potent than bradykinin and at least as potent as synthetic oxytocin (Syntocinon). The threshold dose was about 0·01 ng/ml nutrient fluid and there was a fair dose-response relation. Tachyphylaxis was either absent or moderate. Large doses of alytesin often elicited a tonus increase persisting for hours, in spite of repeated washing with fresh nutrient solution. The effect was atropine-resistant.

There was intense stimulant action on the rat and the guinea-pig colon, as well as on the cat ileum. Tachyphylaxis was absent or moderate and there was again a satisfactory dose-response relation, especially for the guinea-pig colon and cat ileum. Threshold doses ranged between 0·03 and 0·3 ng/ml. Atropine had no appreciable effect. In contrast to the colon, the ileum of the guinea-pig was generally poorly sensitive to alytesin, which produced repeated spikes of contraction, something lasting for hours. Tachyphylaxis was evident and atropine greatly reduced or inhibited the response to the polypeptide.

Alytesin had remarkable stimulant action on the gastric secretion of the chicken and the dog. In the chicken the intravenous infusion of 10–25 ng/kg min^{-1} of alytesin elicited a 50–150% increase in flow of gastric juice and an increase in acid and pepsin outputs. The concentration of pepsin in gastric juice was nearly doubled. Alytesin possessed approximately 5 to 10% of the potency of caerulein. The effect was inhibited by atropine. In dogs provided with denervated gastric pouches alytesin manifested approximately 5% of the action of caerulein and again the secretagogue effect was inhibited by atropine.

By methods measuring transmucosal potential differences and short-circuit current it was shown that alytesin caused an increased transport of Cl^- ions from the serosal to the mucosal surface of the isolated gastric mucosa of *Rana esculenta* and *Bufo viridis*. The threshold concentration was approximately 0·05–0·1 μg/ml of bath fluid. Alytesin was at least as active as pentagastrin, but 500–1000 times less active than caerulein. The effect was vaguely proportional to the dose and atropine-resistant.

There was a moderate hyperglycaemic effect in anaesthetized dogs and rats. In the rat, 15 μg/kg of alytesin given subcutaneously produced a 70% increase of the blood sugar level lasting 2 h while 50 μg/kg produced a 90% increase lasting for more than 5 h. In the dog, the intravenous infusion of 1 μg/kg min^{-1} of alytesin, for 10 min, caused, in addition to hypertension, an increase in blood sugar levels from 120 to 200 mg %, a progressive increase of immunoreactive insulin levels in femoral artery blood (up to 300%) and a 25–30% reduction of blood calcium levels. Return to basal values took 10–30 min.

The following isolated preparations were insensitive or poorly sensitive to alytesin or showed marked tachyphylaxis (in parentheses are the threshold doses per ml of nutrient fluid): rabbit non-pregnant uterus (>0·1 μg/ml), cat post-partum uterus (>0·5 μg/ml); uteri of guinea-pigs and hamsters pretreated with oestrogens (>0·1 μg/ml); rabbit large intestine (0·1 ng/ml, but prompt and intense tachyphylaxis), rabbit duodenum (0·1 ng/ml, but intense tachyphylaxis; occasionally stimulation was preceded by short-lasting inhibition of tone and movements), rat duodenum (no stimulation up to 0·1 μg/ml, doubtful relaxation), chicken terminal ileum and rectal caecum (1–10 ng/ml, no dose-response relation); guinea-pig tracheal chain (>5 μg/ml). The cat, guinea-pig and hamster uteri responded to 2–20 μU oxytocin/ml. The *in situ* gall bladder of the guinea-pig was contracted by intravenous injections of 50–400 ng/kg of alytesin.

Bombesin presented a spectrum of biological activity indistinguishable from that of alytesin. On some preparations it appeared slightly less active. Several fragments of the alytesin and bombesin molecules have been prepared by synthesis or by enzymic hydrolysis, and are being examined.

Nakajima, Tanimura & Pisano (1970) have recently isolated from methanol extracts of the skin of the American frog *Rana pipiens* an endecapeptide, ranatensin, the chemical resemblance of which with alytesin and bombesin appears obvious from the structural formula:

Pyr-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2

In preliminary pharmacological investigation (Geller, Govier & others, 1970) ranatensin manifested an hypertensive effect in dogs and rabbits, but was hypotensive in rats and monkeys. Moreover it stimulated the rat isolated uterus and guinea-pig ileum, and aortic strips of the rabbit. It had no effect on rat aortic strips and relaxed the rat duodenum.

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