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Salivatory effects induced by pirenzepine, atropine, metacine and hexamethonium in preganglionar chronically denervated human parotid gland

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Abstract—Both classical (atropine) and non-traditional (pirenzepine, metacine) antagonists of the muscarinic cholinoreceptors induce, rather than block, an intense and prolonged salivary response in chronically denervated human parotid glands and thus are capable of discriminating between neuronal and aneuronal receptors. Hexamethonium (benzohexonium) a ganglion-blocking agent (0·4 mL, 2·5%) completely inhibits this paradoxical salivation to atropine, benzilylcholine (metacine) and pirenzepine in the chronic preganglionically denervated human parotid gland. The authors discuss the essence of the revealed paradoxical phenomena.

Preganglionic denervation of the otic parasympathetic ganglion (ganglion oticum)—the transmission point of the secretory impulses from the salivary centre of the medulla oblongata to the parotid gland—occurs after trauma to the skull base, fracture of temporal bones and the pyramids or as a result of surgical removal of tumours of the posterior cranial fossa or other surgery in the area of the tympanic cavity (Levin 1948, 1956, 1985, 1986). The post-denervational syndrome of the tympanic nerve or its anastomosis, the minor petrosal nerve (preganglionic branch), is characterized by initiation of an extremely intense and prolonged (up to 4–6 h) salivation in response to minimal and usual doses of cholinolytics (atropine, benzilycholine, scopolamine (Levin 1948, 1985, 1986). For treatment of patients in the acute post-traumatic period and to prevent the remote consequences of trauma, hexamethonium is recommended (Bunatyan & Meshcheryakov 1980). It is also recommended for the prevention of dystrophic disorders in facial muscles arising from concomitant disturbances of the facial nerve (Anichkov 1969). Cholinergic drugs are recommended to prevent gland atrophy and facial muscle deformation. Atropine is also used to show secretory nerve damage, to reveal dynamics in its disturbance and restitution, and in the accompanying vegetative disorders. Pirenzepine is used in patients with gastroenterological disturbances.

Materials and methods

Capsules for saliva collection were attached to both parotid ducts of suitable patients. As a reflectory irritant, 0·5% solution of citric acid (10·0–30·0 mL) was used. Atropine and benzilylcholine solutions (0·1%, 0·5 mL) were injected subcutaneously, and pirenzepine intramuscularly (10 mL) or orally (25 mg). The secretory response to these agents was determined at 60, 90 and 120 min. When the capsules were removed to avoid overstraining the patients, salivation continued for several hours. Hexamethonium was used subcutaneously (2·5%, 0·4 mL) or azamethonium bromide (pentamine) (5·0%, 0·4 mL) was introduced intramuscularly. Hexamethonium was injected 5–7 min before the atropine and pirenzepine or, more often, 5–7 min after initiation of vigorous atropine secretion, or at different times after initiation of the intense salivation.

Eight subjects were observed. In five subjects (4 men, 1 woman), aged 20 to 54 years, the atropine salivatory paradox resulted from cerebrocranial trauma, while in three women, aged 27 to 47 years, it appeared after surgery for removal of the acoustic nerve neurinoma.

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Results

Hexamethonium or azamethonium injection after 5–10 min resulted in a complete blockade of the paradoxical secretion to atropine, or primarily in its sharp inhibition and then cessation. With complete blockade of the paradoxical secretion, trigger irritation with citric acid was applied without response which proved the stability of the blockade of atropine secretion (Table 1).

Regardless of the time of injection—before atropinization, immediately after the beginning of the paradoxical secretion, or during the secretion—gangliolytics immediately stopped the phenomenon.

Repeated atropine injection did not reverse the hexamethonium-induced blockade, while hexamethonium itself did not stimulate salivation from the denervated gland. At the same time, citric acid did not excite reactivation of the paradoxical secretion even 2 h after injection of hexamethonium. Hexamethonium (2·5%, 0·4 mL) did not inhibit reflectory and pilocarpine-induced secretion from the intact gland or the denervated gland.

Table 2 summarises the findings on application of the non-traditional antimuscarinic agents metacine and pirenzepine. The level and duration of the secretory effects of these agents were similar to the atropine effects (Table 1). Hexamethonium was also capable of suppressing the paradoxical salivation caused by these cholinolytics.

Application of amitriptyline, which is structurally similar to pirenzepine (except for the presence of an oxygen in the side chain), did not induce paradoxical secretion (results from three subjects). None of the agents used caused salivation from intact (control) gland.

Fig. 1 demonstrates secretion levels in a patient following administration of pirenzepine (25 mg) orally; salivation started 50 min after taking the preparation. Subcutaneous or intramus-

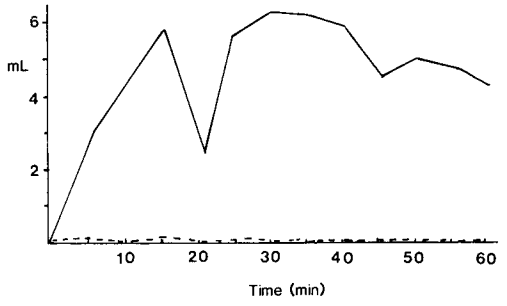

FIG. 1. Demonstration of paradoxical salivation to pirenzepine (25 mg administered orally). Salivation initiated 50 min after taking the preparation. Secretion continued for 2·5 h more after the removal of the collection capsules. ——— Denervated gland, - - - - - Intact gland.

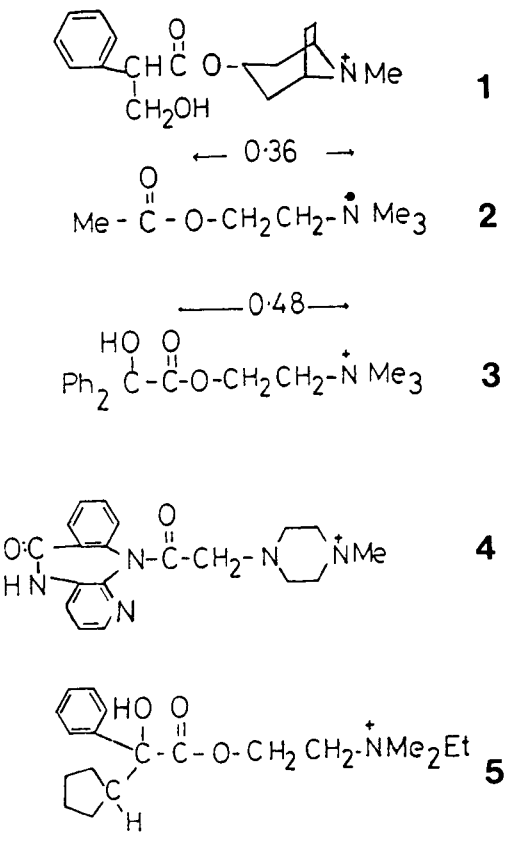

FIG. 2. Agents used. 1. Atropine. 2. Acetylcholine. 3. Benzilylcholine. 4. Pirenzepine. 5. Chlorosyl.

As the formulae show, all the ligands have a strict sterically-specified side chain which provides complementary binding at the receptor anionic pole. The composition and length of the accessory groups ('hydrophobic tails') differ among the various ligands, which points to a lack of strictly restricted, specified sites in the domains with which these additional rings interact. In other words, there is a strict spatial restriction for a side chain and 'blurred' limits for weighted and various additional radicals at the esterophilic receptor pole.

The absence of 'hydrophobic tails' in cholinomimetics normally accounts for their cholinopositive activity, while their presence in cholinolytics normally determines the cholinonegative effect. However, after chronic denervation of the gland the modified ligands change their action and become transformed from antagonists into agonists. According to current thinking, denervation results in the generation of newly synthesized receptors with new genetic determinants which condition other chemical, physiological, pharmacological or immunological properties (Grampp et al 1972; Fambrough 1979; Merlie et al 1984).

* Note: Direct atom to atom distances, which may be more relevant than chain length, calculated for the five molecules are as follows:

Compound	N^+ to carbonyl oxygen distance (nm)	N^+ to ether oxygen distance (nm)
Atropine	0·612	0·425
Acetylcholine	0·499	0·337
Benzilylcholine	0·573	0·410
Pirenzepine	0·543	—
Chlorosyl	0·513	0·383

According to Mishina et al (1986), embryonic muscarinic cholinoreceptors (M-CR) have a molecular mass of 86000 daltons, and mature ones 72000 daltons. During maturation of M-CR, the γ sub-unit is exchanged for an ζ subunit (Takai et al 1985). Normally, the presence of accessory cyclic groups enables the generation of additional bonds (hydrophobic, hydrogen or Van der Vaals) which during the action of cholinolytics fix the receptor in the inactive state preventing the action of agonists.

It is therefore reasonable to suggest that, after chronic denervation, the extra groups in the antimuscarinic drugs, while interacting with other fragments in the aminoacid chain of receptors, are now incapable of fixing the receptors in the inactive state or promoting a highly intense cholinopositive response.

Some authors (Wheatley et al 1988) believe that the positively charged acetylcholine ammonium grouping is bound to the negatively charged asparagine 71 and 105 in the aminoacid chain of the second and third transmembrane domains. Within these groups, the activity of G-proteins is seen. The esterophilic ACh moiety associates with serine of the third domain. The accessory groups of antagonists enter the interspiral gaps of the hydrophobic fragments in the fourth, fifth and sixth domains and become rigidly fixed in these narrow formations, thus inhibiting the conformational modifications of the receptor and its metabolically productive transformations.

We suggest that, after denervation, the hydrophobic fractions of domains in the newly synthesized receptors are replaced by amphipathic and hydrophilic aminoacids, or chains in these acids are displaced to the extra- or intraposition with regard to the plane of the plasmotic membrane. We also suggest that the degree of spiralization in the protein structure is incapable of providing solidity in anchoring the 'hydrophobic tails' to corresponding sites.

The weak attraction of the cyclic rings to the diffuse ester group would permit the relatively free ammonium end of the ligand to react with the active centres of the receptor and initiate reversible productive conformational transformations of the receptor, thereby causing the prolonged nature of the atropine effect of scopolamine, benzilylcholine and pirenzepine as agents that are not hydrolysed by cholinesterase.

The paralysing effect of hexamethonium on paradoxical salivation initiated by atropine, benzilylcholine and pirenzepine requires a separate study. From the work of Gurney & Rang (1984) and Skok (1985), pointing to the effect of hexamethonium within the ionic canals, we suggest that, while acting within the ionic canals at the remote periphery of the device effectors, hexamethonium can inhibit the paradoxical responses to aggravated cholinergic ligands.

ADDENDUM

Pauling & Petcher (1970) defined the crystal structure of atropine and compared it with that of acetylcholine (ACh). The affinity of conformational structures of these molecules in the crystalline state allowed them to conclude that atropine's analogues and ACh associate with muscarinic cholinoreceptors (M-CR) in the same manner with the same areas of the receptor. Using NMR it was established that ACh and its analogues have the same synclial O-N gauche-structure in solution as in the crystalline state (Feeney et al 1977).

X-ray crystallographic studies show that anticholinergic substances are formally derivatives of ACh, with the acyl group cyclised and ethyl groups introduced into the ring system (Guy & Hamor 1975).

The conformation and spatial orientation of pirenzepine molecules (and probably of its analogue telenzepine) resemble the conformation of 24 known anticholinergic agents which are

defined by X-ray structural analysis and other methods (Pauling & Datta 1980; Trummlitz et al 1984). Though pirenzepine and telenzepine are tricyclic compounds, the above mentioned similarity to the crystalline conformation of the known tricyclic antidepressants, neuroleptics and antihistamine agents is absent.

The structural similarity with elements of the molecules of classical cholinolytics (atropine, scopolamine, quinuclidinyl benzilate) relates pirenzepine to both M-CR and the functional antimuscarinic effect of these ligands.

The hydrogen atom of the amide group of pirenzepine and telenzepine has the same function as hydrogen or hydroxyl of atropine, scopolamine and quinucleodinyl benzilate. The intramolecular distances in the active conformation of pirenzepine and telenzepine also resemble those of classical antimuscarine ligands. The calculated potential forces of their electronic fields provide both selected binding in-vitro and a functional activity in-vivo relating to M-CR. Although pirenzepine and telenzepine are not classical antagonists of acetylcholine receptors, the above explains their role as antimuscarine ligands. As atropine, scopolamine and their derivatives play the role of agonists with reference to the chronically denervated human parotid gland, it is quite clear that agents having the likely stereospecific conformation play a similar role to M-CR activators deprived of neural control.

The question why, after denervation of this gland, the ACh antagonists should lose their normal function as antagonists, may be answered only by reference to the evolution of the M-CR system.

References

Anichkov, S. V. (1969) Neurogenic dystrophies and pharmacotherapy. (In Russian) Leningrad: Medgiz Publ.

Bunatyan, A. A., Meshcheryakov, A. V. (1980) Ganglion blocking agents in anaesthesiology.—In Kharkevich, A. D. (Ed.) Pharmacology of Ganglionic Transmission. Springer, New York pp 439–463

Fambrough, D. M. (1979) Control of acetylcholine receptors in skeletal muscle. Physiol. Rev. 59: p. 165–227

Feeney, J., Foster, R., Piper, E. A. (1977) Nuclear magnetic resonance study of the conformation of atropine and scopolamine cations. J. Chem. Soc. Perkin Trans. 2: 2016–2020

Grampp, W., Harris, J. B., Thesleff, S. (1972) Inhibition of denervation changes in skeletal muscle by blockers of protein synthesis. J. Physiol. 221: 743–754

Gurney, A. M., Rang, H.P. (1984) The channel-blocking action of methonium compounds of rat submandibular ganglion cells. Br. J. Pharmacol. 82: 623–642

Guy, J. J., Hamor, T. A. (1975) Stereochemistry of anticholinergic agents. Part VI: Crystal and molecular structure of hexamonium iodide. J. Chem. Soc. Perkin Trans. 2: 464–471

Levin, S. L. (1948) Atropine as a stimulator of salivation. (In Russian) Bull. Exp. Biol. Med. USSR 25: 280–283

Levin, S. L. (1956) Some data concerning salivation of the partially denervated human parotid gland. (In Russian) J. Physiol. USSR 42: 390–397

Levin, S. L. (1985) Effect of denervation on parotid gland function. Oto-Rhyno-Laryngol. Dig., June: 29–30

Levin, S. L. (1986) Dualism in the effect of cholinolytics upon the human parotid gland deprived of parasympathetic control. Int. J. Clin. Pharmacol. Res. 6: 429–439

Merlie, J. P., Isenberg, R. E., Russel, S. D., Sanes, J. R. (1984) Denervation supersensitivity in skeletal muscle: analysis with a clonex cDNA probe. J. Cell Biol. 99: 332–335

Mishina, M., Takai, T., Imato, K., Noda, M., Takahashi, T., Numa, S., Methfessel, C., Sakmann, B. (1986) Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature 321: 402–411

Pauling, P., Datta, N. (1980) Anticholinergic substances: a single consistent conformation. Proc. Natl. Acad. Sci. USA (Chemistry) 77: 708–712

Pauling, P. J., Petcher, T. J. (1970) Interaction of atropine with the muscarinic receptor. Nature 228: 673–674

Skok, V. I. (1985) Localization and structure of connection areas of bis-ammonium compounds in nicotinic cholinoreceptor. Biol. Membranes 2: 345–355 (In Russian)

Takai, T., Noda, M., Mishina, M., Shimizu, S., Furatami, V., Kagano, T., Ikeda, T., Kubo, T., Takahashi, T., Kuno, M., Numa, S. (1985) Cloning sequencing and expression of cDNA for a novel subunit of acetylcholine receptor from calf muscle. Nature 315: 761–764

Trummlitz, G., Schmidt, G., Wagner, H. U., Luger, P. (1984) Conformational analysis of the antiulcer drug pirenzepine. Arzneimit. Forsch. 34: 849–859

Wheatley, M., Hulme, E. C., Birdsall, N. J. M., Curtis, C. A. M., Eveleigh, P., Pedder, E. K., Pogner, D. (1988) Peptide mapping studies on muscarinic receptors: receptor structure and location of the ligand binding site. TIPS Suppl. Subtypes of Muscarine Receptors, III, pp 19–24