Halpern, B. N., Musso, E., Neveu, T. (1955) Action of the histamine-releaser polyvinylpyrrolidone on capillary permeability in dogs. Br. J. Pharmacol. 10: 223–229

Lichtenstein, L. M., Gillespie, E. (1975) The effects of the H1 and H2 antihistamines on "allergic" histamine release and its inhibition by histamine. J. Pharmacol. Exp. Ther. 192: 491–450

Lorenz, W., Barth, H., Thermann, M., Schmal, A., Dormann, P., Niemeyer, I. (1974) Fluorometric histamine determination in canine plasma under normal conditions, following application of exogenous histamine, and during histamine release by Haemaccel. Hoppe-Seyler's Z. Physiol. Chem. 355: 1097–1111

Paton, W. D. M. (1957) Histamine release by compounds of simple chemical structure. Pharmacological Reviews 9: 269–328

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Letter to the Editor

Muscarinic receptor subtypes involved in bethanechol-induced water intake in the rat

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We have recently described a compulsive drinking behaviour elicited by bethanechol through activation of central muscarinic receptors in the rat (Schiavone et al 1987). The bethanechol-induced water intake was specifically inhibited by some antimuscarinic drugs, while being insensitive to histaminergic (H_1 and H_2), adrenergic (α and β) and 5-hydroxytryptaminergic antagonists.

The present report attempts to characterize the drinking response to bethanechol in relation to the muscarinic receptor subtype involved.

The method employed was that of Schiavone et al (1987). Briefly, water intake was measured for 30 min following injection of bethanechol, 10 μg/rat, into the right cerebral ventricle through a chronically implanted cannula. The effect of antagonists was evaluated after their intraventricular administration, 2 min before bethanechol challenge.

We used pirenzepine and AF-DX 116 as muscarinic antagonists selective for M_1 and M_2 (cardiac) receptors, respectively. Those compounds have provided the biochemical and functional basis for the differentiation of muscarinic receptors into subtypes (see reviews by Hirshowitz et al 1984; Levine et al 1988).

Results showed that both compounds were able to inhibit bethanechol-induced water intake, albeit with different potencies. The ID50 (and 95% confidence limits), expressed in pmol/rat, were 110 (18–700) and 1800 (610–5400) for pirenzepine and AF-DX 116, respectively.

Thus, when compared with the ID50 of the non-selective drug atropine, 6·7 pmol/rat (Schiavone et al 1987), the potencies of pirenzepine and AF-DX 116 were 17- and 270-fold lower, respectively. The potency of AF-DX 116 in some functional responses subserved by the cardiac muscarinic receptor, i.e. the subtype recognized as having high affinity for the drug, has been shown to be about one order of magnitude lower than that of atropine (Giachetti et al 1986; Micheletti et al 1987). The potency ratio found in the present study (270) falls in the range of magnitude (two to three orders) typical of effects due to receptors recognized by AF-DX 116 as having low affinity (Micheletti et al 1987). On this evidence, that the receptor activated by bethanechol belongs to the M_2 (cardiac) subtype can be confidently excluded.

Conversely, pirenzepine was about 1/20th as potent as atropine in inhibiting compulsive drinking. Despite the noticeable potency of pirenzepine, the receptor stimulated by bethanechol cannot be unambigously defined as M_1 for two main reasons: i) the lower lipophilicity of pirenzepine in comparison to atropine might increase the apparent efficacy of the former drug by slowing its diffusion rate from the active compartment. In this context it is worth mentioning that the order of lipophilicity is: AF-DX 116 > atropine > pirenzepine (log P at pH 12–14 being +2·2, +1·5, +0·1, respectively; Engel, personal communication); ii) cloning and sequencing of muscarinic receptor genes from pig brain (Kubo et al 1986a) and heart (Kubo et al 1986b; Peralta et al 1987) and rat brain (Bonner et al 1987) has demonstrated the existence of four separate receptor molecules. Three of these functional genes are expressed in the rat cerebral cortex, and are not clearly distinguished by pirenzepine, which binds them with homogeneous high affinity (Bonner et al 1987). It is therefore conceivable that anyone of the three subtypes is responsible for the bethanechol effect.

Recently, Hagan et al (1987) speculated that cholinergic stimulation of drinking from the lateral hypothalamus was mediated by M_2 receptors. This conclusion was based on the inefficacy of putatively selective M_1 agonists (McN-A-343, AHR 602, AH 6405) in promoting drinking, and on the lack of a systematic relationship between antagonist potency and their affinity for the M_1 receptor. Since McN-A-343 and related compounds do not discriminate among muscarinic subtypes as regards affinity, their lack of efficacy as drinking-inducers may depend on a small receptor number, presumably inadequate for partial agonists (Eglen & Whiting 1986). Interestingly, M_1 receptors represent only a small proportion of the total muscarinic receptors (Cortes & Palacios 1986) in brain regions from which a drinking response was elicited by direct intracranial injection of carbachol in the rat (Swanson & Sharpe 1973).

In conclusion, the receptor subtype responsible for muscarinic-induced water intake in the rat cannot presently be clearly identified other than as non M_2 (cardiac). The advent of more selective agents should help to clarify this issue.

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References

Bonner, T. I., Buckley, N. J., Young, A. C., Brann, M. R. (1987) Identification of a family of muscarinic acetylcholine receptor genes. Science 237: 527–532

Cortes, R., Palacios, J. M. (1986) Muscarinic cholinergic receptor subtypes in the rat brain. I. Quantitative autoradiographic studies. Brain Res. 362: 227–238

Eglen, R. M., Whiting, R. L. (1986) Muscarinic receptor subtypes: a critique of the current classification and a proposal for a working nomenclature. J. Auton. Pharmacol. 5: 323–346

Giachetti, A., Micheletti, R., Montagna, E. (1986) Cardioselective profile of AF-DX 116, a muscarinic M_2 receptor antagonist. Life Sci. 38: 1663–1672

Hagan, J. J., Tonnaer, J. A. D. M., Broekkamp, C. L. E. (1987) Cholinergic stimulation of drinking from the lateral hypothalamus: indications for M_2 muscarinic receptor mediation. Pharmacol. Biochem. Behav. 26: 771–779

Hirschowitz, B. I., Hammer, R., Giachetti, A., Keirns, J. J., Levine, R. R. (1984) Proceedings of the international symposium on subtypes of muscarinic receptors. Trends in Pharmacol. Sci. 5 supplement

Kubo, T., Fukuda, K., Mikami, A., Maeda, A., Takahashi, H., Mishina, M., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., Numa, S. (1986a) Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. Nature 323: 411–416

Kubo, T., Maeda, A., Sugimoto, K., Akiba, I., Mikami, A., Takahashi, H., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Matsuo, H., Hirose, T., Numa, S. (1986b) Primary structure of porcine cardiac muscarinic acetylcholine receptor deduced from the cDNA sequence. FEBS Lett. 209: 367–372

Levine, R. R., Birdsall, N. J. M., North, H. R., Holman, M., Watanabe, H., Iversen, L. L. (1988) Proceedings of the Third International Symposium on Subtypes of Muscarinic Receptors. Trends in Pharmacol. Sci. 9 supplement

Micheletti, R., Montagna, E., Giachetti, A. (1987) AF-DX 116, a cardioselective muscarinic antagonist. J. Pharmacol. Exp. Ther. 241: 628–634

Peralta, E. G., Winslow, J. W., Peterson, G. L., Smith, D. H., Ashkenazi, A., Ramachandran, J., Schimerlik, M. I., Capon, D. J. (1987) Primary structure and biochemical properties of an M_2 muscarinic receptor. Science 236: 600–605

Schiavone, A., Prudentino, A., Giachetti, A. (1987) Central muscarinic activation elicits compulsive drinking behaviour in the rat. J. Pharm. Pharmacol. 39: 304–306

Swanson, L. W., Sharpe, L. G. (1973) Centrally induced drinking: comparison of angiotensin II- and carbachol-sensitive sites in rats. Am. J. Physiol. 225: 566–573