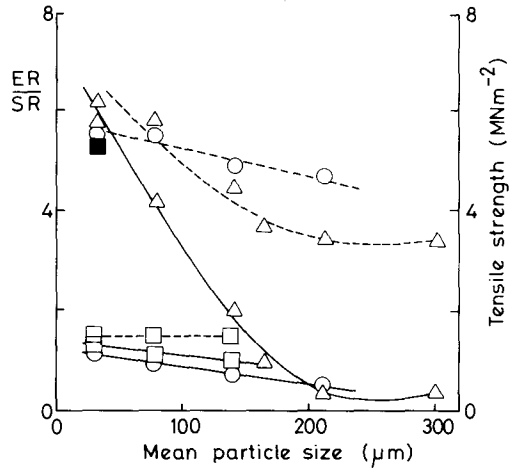


FIG. 2. Effect of particle size on ER/SR and tensile strength for Emcompress at $\rho_f = 0{\cdot}74$, lactose at $\rho_f = 0{\cdot}85$ and on the tensile strength of Sta-Rx at $\rho_f = 0{\cdot}85$ and Avicel at $\rho_f = 0{\cdot}81$. - - - - ER/SR, —— Tensile strength, △——△ Emcompress, ○——○ Lactose, □——□ Sta-Rx, ■ Avicel.

not for Sta-Rx whose value remained approximately constant at 1·5 (Fig. 2). No explanation can be offered for the greater effect of particle size on ER/SR for Emcompress than for the other materials.

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# Central muscarinic activation elicits compulsive drinking behaviour in the rat

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Injection of bethanechol into the lateral cerebral ventricle of the rat induces a marked increase in drinking, within 30 min from administration. The response is dose-related, maximal water intake (6·1 ± 0·55 mL; mean ± s.e.) occurring at 10 μg of bethanechol. Peripheral administration of the agonist (up to 3 mg kg$^{-1}$ i.p.) fails to elicit drinking. Among several specific antagonists only anti-muscarinic drugs produced a significant inhibition of the response, suggesting that the compulsive drinking behaviour in the rat is caused by activation of central muscarinic receptors. The drinking behaviour emerges as a reliable test to assess central muscarinic activity of both agonists and antagonists.

Central cholinergic stimulation by carbachol induces a short latency drinking response in the rat (Grossman 1960; Swanson & Sharpe 1973; Hoffman & Phillips 1977; Menani et al 1984). A similar effect is also exhibited by angiotensin II (Epstein et al 1970; Swanson & Sharpe 1973; Hoffman & Phillips 1977), acting, however, through an independent peptidergic pathway, as demonstrated by its sensitivity to the specific antagonist, saralasin (Giardina & Fisher 1971; Hoffman & Phillips 1977). Whether central muscarinic receptors activated by carbachol are solely responsible for the dipsogenic response remains uncertain, since hexamethonium, applied to the subfornical area, produces a partial blockade (Menani et al 1984). Moreover, nicotine, when applied centrally, causes a moderate but measurable increase of water intake (Stein & Seifter 1962). To avoid possible direct nicotinic stimulation we have studied the drinking behaviour induced by intraventricularly (i.vt.) applied bethanechol, a muscarinic agonist devoid of the nicotinic stimulating properties of carbachol, yet resistant to hydrolysis by cholinesterases (Taylor 1985). In addition we have tested specific antagonists of different receptor systems for their effect on bethanechol-induced water intake.

* Correspondence.

*Materials and methods*

Male Sprague-Dawley (Charles River) rats, 200–250 g, were used. Under sodium pentobarbitone (30 mg $kg^{-1}$ i.p.) anaesthesia, a PE 10 cannula was implanted into the right lateral cerebral ventricle (Green et al 1976) (2 mm lateral, 2 mm posterior to the bregma and 4 mm below the surface of the skull). The cannula was fixed to the skull with screws and acrylic resin. Rats were individually housed under controlled environmental conditions with free access to food and water. A one week recovery period allowed the animals to become accustomed to drinking from calibrated burettes (0·2 mL markings) provided with metal spouts. Experiments were performed in the morning (1000 h), while food was withheld. Water intake was measured during 30 min after i.vt. injection of bethanechol dissolved in 5 μL saline (NaCl 0·9%). The dose of bethanechol (10 μg/rat, 50·8 nmol), which under our experimental conditions induced maximal drinking response (Fig. 1), was chosen to study the effect of various antagonists. The latter, dissolved in saline, were administered either i.vt. (5 μL) 2 min, or intraperitoneally (i.p., 0·5 mL/100 g) 15 min before bethanechol. The doses of antagonists administered i.vt. represent total amount per rat.

For each antagonist, 3 to 4 dose levels were examined (n = 4–6); a group receiving only saline before bethanechol challenge served as control.

At the end of a series of experiments the position of the cannula was verified histologically; when the position was dubious, data were discarded.

ID50, defined as the dose of antagonist reducing by 50% the mean water consumption of the relative control group, and their 95% confidence limits (c.l.) were calculated using a least squares linear regression analysis by plotting the individual water intake values against log doses. Statistical significance was evaluated using a random anova, except for data reported in Table 2 where Student's *t*-test was applied.

The drugs used were: pentobarbitone sodium (Seigfried, Zofingen, Switzerland), bethanechol HCl, mepyramine maleate, propranolol HCl, methylatropine methylbromide (Sigma, St Louis, MO, USA), atropine sulphate (BDH, Poole, Dorset, UK), benzhexol HCl (Serva, Heidelberg, W. Germany), dexetimide HCl (Janssen, Beerse, Belgium), hyoscine bromide (B.H. Schilling, Milan, Italy), methysergide hydrogenmaleate (Sandoz, Basle, Switzerland), phentolamine methanesulphonate (Ciba-Geigy, Basle, Switzerland), propantheline methylbromide, cimetidine (Gianni, Milan, Italy).

*Results*

Intraventricular injection of bethanechol induced a dose-related increase in water consumption within the 1–10 μg range (Fig. 1). A detectable water intake was observed at 1 μg (5·08 nmol), while maximal water intake (6·10 ± 0·55 mL; mean ± s.e.; n = 10) was obtained with 10 μg (50·8 nmol). Further increase in the bethanechol dose produced a slight reduction in drinking. The latency of the drinking response was short, approaching 1 min at higher doses of agonist. General behaviour of the rat was not affected by any dose tested.

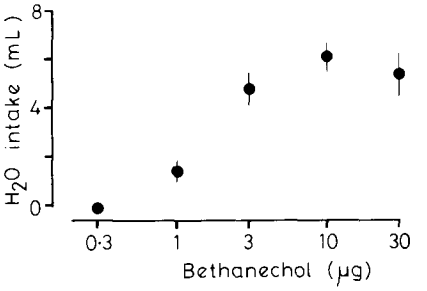


FIG. 1. Dose-response curve for drinking produced by i.vt. bethanechol. Each point is the mean ± s.e. of 10 animals. Response was linearly related to the dose administered between 1 and 10 μg, regression line calculated on these points was highly significant ($P < 0·01$, random anova test).

Intraperitoneal administration of bethanechol failed to increase water consumption (up to 3 mg $kg^{-1}$; 15·2 μmol $kg^{-1}$), although overt symptoms of muscarinic excitation (diarrhoea, salivation, chromodacryorrhoea) were evident.

Several muscarinic antagonists administered by i.vt. and i.p. routes inhibited the bethanechol-induced water intake in a dose-related fashion; their ID50s and 95% c.l. are reported in Table 1.

Table 1. Inhibition by muscarinic antagonists of bethanechol-induced water intake.

| Drug | ID50 (95% c.l.) i.vt. (pmol/rat) | ID50 (95% c.l.) i.p. (μmol $kg^{-1}$) | Ratio i.p./i.vt. × $10^3$ |
|---|---|---|---|
| Atropine | 6·7 (3·9 – 11·6) | 1·0 (0·6 – 1·8) | 149 |
| Propantheline | 9·8 (4·9 – 19·8) | 5·9 (3·6 – 10·6) | 602 |
| Methylatropine | 4·4 (2·6 – 7·2) | 4·6 (1·5 – 14·0) | 1045 |
| Benzhexol | 82·6 (33·5 – 203·4) | 0·4 (0·13 – 0·9) | 5 |
| Dexetimide | 9·1 (2·6 – 32·3) | 0·4 (0·14 – 1·2) | 44 |
| Hyoscine | 3·4 (0·9 – 13·0) | 0·04 (0·01 – 0·17) | 12 |

ID50s with 95% confidence limits were calculated from regression lines with 3–4 doses. Each dose 4–6 animals.

All drugs displayed a remarkable activity, inhibiting the response in the picomolar range after i.vt. administration. When administered i.p., the inhibitory potency of muscarinic antagonists was in a wider range than that observed after i.vt. injection.

The ratios between ID50 after i.p. and i.vt. administration, reported in Table 1, are indicative of the ability of the drug to cross the blood-brain barrier.

The lowest ratio was found for benzhexol, a drug known to exert prominent central effects. As expected,

methylatropine was characterized by the highest ratio.

To verify the specificity of muscarinic-mediated drinking, a number of antagonists active at different receptor systems were tested (Table 2). Some inhibition was observed at doses of antagonist far in excess of those needed to block fully their own receptor system. Histamine $H_1$ and $H_2$ antagonists, typified by mepyramine and cimetidine, appeared to enhance the drinking response although this occurred at large i.vt. doses of either drug.

Table 2. Bethanechol-induced water intake. Effect of specific antagonists injected intraventricularly.

| Drug | Dose (nmol/rat) | mL water intake | |
|---|---|---|---|
| | | Treated | Control |
| Hexamethonium | 42 | 3·15 ± 0·47 | 5·84 ± 1·49 |
| Mepyramine | 35 | 11·12 ± 0·99* | 7·08 ± 1·46 |
| Cimetidine | 119 | 9·40 ± 2·05 | 7·08 ± 1·46 |
| Phentolamine | 36 | 3·75 ± 2·03 | 7·92 ± 1·61 |
| Propranolol | 39 | 4·96 ± 1·53 | 7·92 ± 1·61 |
| Methysergide | 28 | 2·10 ± 0·74 | 4·30 ± 1·24 |

Each result is the mean of 4–6 animals.
* Significantly different from control $P < 0·05$ (Student's $t$-test).

*Discussion*

Bethanechol, applied directly into the cerebral ventricles of rats, elicits compulsive drinking behaviour characterized by short latency. The magnitude of its response is comparable with that reported for carbachol, although the latter agonist appears to be 25 to 50 times more potent in causing a dipsogenic effect (Giardina & Fisher 1971; Hoffman & Phillips 1977; Sumners et al 1981). Muscarinic agonists appear to stimulate brief but intense thirst in the rat, as the water consumed during the experiment (30 min) represents 25–30% of the daily fluid intake.

A central locus of action for bethanechol is indicated by two observations: (i) its inability to induce a response when administered intraperitoneally; (ii) the large difference in potency between centrally and peripherally administered methylatropine, a drug which poorly penetrates the blood-brain barrier.

As bethanechol is devoid of nicotinic effects (Taylor 1985), its response may be attributed to specific activation of muscarinic receptors at central sites. The trivial inhibition exerted by hexamethonium after large i.vt. dose, is unspecific and thus excludes involvement of nicotinic activation. In keeping with the muscarinic-mediated effect of bethanechol are the results obtained with muscarinic antagonists injected i.vt. In general, their potency in suppressing water intake parallels that seen in in-vitro assays (Inch & Brimblecombe 1974). The rank order of activity after i.p administration does not reflect their antimuscarinic potency, but rather their propensity to cross the blood-brain barrier.

The specificity of bethanechol-induced drinking is illustrated by the poor efficacy of non-selective antagonists to interfere with the response. Thus, the drinking behaviour appears to be a reliable test, useful in evaluating central muscarinic activity of both agonists and antagonists. In addition, the differences observed between i.p. and i.vt. potencies of a series of antimuscarinics represent a means of assessing a compound's ability to penetrate the blood-brain barrier. Experiments designed to detect drugs interfering with the central muscarinic transmission are of interest for their potential application in Alzheimer's and related diseases, in which a cholinergic deficit has been reported.

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