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CHOLINERGIC ACTION IN THE NUCLEUS ACCUMBENS: MODULATION OF DOPAMINE AND ACETYLCHOLINE RELEASE

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1 The action of oxotremorine and acetylcholine on the release of dopamine and acetylcholine from tissue slices of the rat nucleus accumbens was studied.

2 Oxotremorine significantly enhanced the release of [^{14}C]-dopamine evoked by 34 mM K^+ and the EC_{50} for this action was 1.5×10^{-7} M. A maximal enhancement (30%) for this effect was reached at 2×10^{-7} M oxotremorine. A further enhancement of dopamine release occurred at concentrations of oxotremorine greater than 10^{-4} M.

3 The action of oxotremorine on [^{14}C]-dopamine release was calcium-dependent and blocked by atropine (10^{-4} M) but not mecamylamine (up to 10^{-4} M).

4 Oxotremorine affected [^{3}H]-acetylcholine release differentially, inhibiting the K^+-evoked release of [^{3}H]-acetylcholine at concentrations greater than 10^{-5} M. The IC_{50} for this process was 4.3×10^{-5} M.

5 Acetylcholine (8×10^{-4} M) showed a similar pattern of action to oxotremorine: it enhanced the K^+-evoked release of [^{14}C]-dopamine (50%) and inhibited the K^+-evoked release of [^{3}H]-acetylcholine (30%).

6 The mechanism of action of oxotremorine on dopamine release is discussed in terms of a presynaptic receptor-mediated process.

Introduction

High concentrations of acetylcholine, choline acetyltransferase and dopamine are found in the nucleus accumbens (Dahlstrom & Fuxe, 1965; Shute & Lewis, 1967; Cheney, Le Fevre & Racagni, 1975). An interaction appears to occur between these transmitters in this region since the cholinergic agonist, oxotremorine, and physostigmine, elevate levels of homovanillic acid *in vivo* (Andén & Bedard, 1971; Bartholini, Keller & Pletscher, 1975). This relationship is likely to occur directly (in the nucleus accumbens) since intra-accumbens injections of carbachol and oxotremorine initially enhance the locomotor hyperactivity induced by bilateral injections of dopamine into the nucleus accumbens (Jones, Morgenson & Wu, 1981; de Belleroche, Herberg, Winn, Murzi & Williams, 1981a). A direct action of acetylcholine on dopaminergic transmission could be mediated via presynaptic cholinoceptors on dopamine terminals. Studies of the specific binding of muscarinic agonists and antagonists do indeed indicate that this could be the case, since the concentration of muscarinic receptors in the nucleus accumbens is depleted following 6-hydroxydopamine injection in the ventral tegmental area (de Belleroche, Kilpatrick, Birdsall & Hulme, 1981).

The aim of this project was to elucidate the action of cholinergic agents on dopamine release in the nucleus accumbens.

Methods

Female Sprague-Dawley rats (200–250 g body wt.) were stunned and killed by cervical dislocation. Two coronal cuts were made at right angles to the axis of the brain. The first cut was made 1 mm anterior to the optic chiasma and the second cut was 1.5 mm anterior to the first cut. The nucleus accumbens was dissected out from this section, a rat brain atlas (König & Klippel, 1963) being used for reference. Four tissue slices of the dissected nucleus accumbens were cut in a plane parallel to the section (0.35 mm thickness, approximately). The tissue slices were immediately immersed in Krebs-bicarbonate medium of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2,

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$CaCl_2.6H_2O$ 2.5, glucose 11.1, ascorbate 2.3 and pargyline 1, pH 7.4 gassed with 95% O_2: 5% CO_2. Tissue slices were incubated in Krebs-bicarbonate medium (2 ml) containing 0.065 μM [methyl-^{3}H]-choline chloride (77 Ci/mmol) and 4.4 μM [7-^{14}C]-dopamine (56 mCi/mmol) at 37°C for 30 min. The tissue slices were then transferred to a perspex block containing 8 tissue chambers and were superfused with Krebs-bicarbonate medium, maintained at 37°C, at a rate of 1 ml/min for a further 30 min. The initial perfusion period was carried out in order to reach a steady and constant base line rate of release. At the end of this period, tissue was treated differently for studying the effect of either oxotremorine or acetylcholine.

Effect of oxotremorine

Tissue slices were transferred with fine forceps to Krebs-bicarbonate medium (1 ml) and incubated for a 10 min period at 37°C under control conditions or in the presence of drug (oxotremorine, atropine, mecamylamine) as indicated. The slices obtained from one nucleus accumbens were divided equally between control and test incubations. The tissue slices were then transferred to fresh medium containing 34 mM K^+ for a 5 min incubation period at 37°C under control or test conditions (drug present) as before. The tissue slices were removed and aliquots (0.8 ml) from the two incubation periods were taken for analysis of the ^{14}C and tritium content using double-label liquid scintillation counting. Aquasol 2 (New England Nuclear Chemicals, Massachusetts) was used as the scintillant. The ^{14}C and tritium content of the tissue slices were also determined following solubilization in Soluene 350 (Packard Instrument Company Inc., Illinois). Purification of the [^{3}H]-acetylcholine and [^{14}C]-dopamine was not carried out as these conditions have previously been found to yield [^{14}C]-dopamine as the main ^{14}C-labelled constituent (90%) of the samples and [^{3}H]-acetylcholine, the main tritium labelled constituent when physostigmine is contained in the medium (de Belleroche, unpublished observations; de Belleroche & Neal, 1981). Efflux of [^{3}H]-acetylcholine or [^{14}C]-dopamine is expressed as the amount released in each incubation period as a percentage of the total remaining (released plus tissue content) at the end of that incubation period divided by the time of the incubation in minutes (e.g. % tissue [^{3}H]-acetylcholine released/min). Collection of samples by superfusion was not considered necessary for the oxotremorine experiments as a marked and significant response was obtained at low concentrations of oxotremorine. However, it was considered important in the experiments on the effect of acetylcholine (next paragraph), where rapid inactivation of acetylcholine by the action of acetylcholinesterase occurs, to attempt to maintain the external acetylcholine concentration by the continuous superfusion method.

Effect of acetylcholine

After the initial superfusion period, collection of superfusate samples (2.4 min) was started. A K^+ pulse was applied by superfusion with oxygenated medium containing 34 mM KCl for 4.8 min. A second K^+ pulse containing acetylcholine (8.2×10^{-4} M) and physostigmine (10^{-4} M) was applied after 12 min of superfusion with control medium, and a third pulse of K^+ (acetylcholine and physostigmine absent) was applied after a similar period (12 min) of superfusion with control medium. The ^{14}C and tritium content of each superfusate sample and the tissue slices after superfusion were determined as described above.

Transmitter release in serial samples is expressed

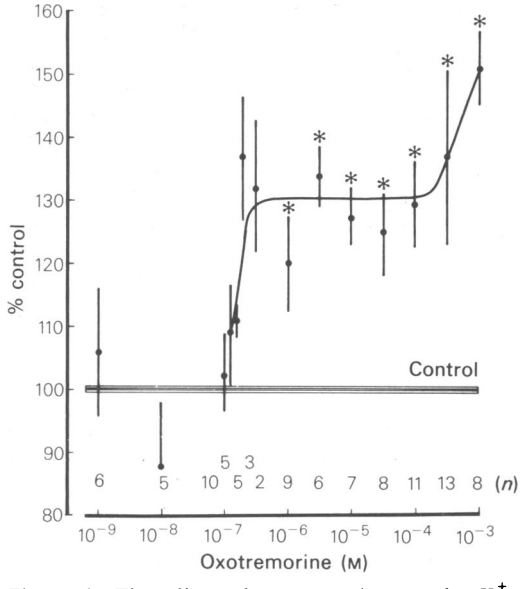

Figure 1 The effect of oxotremorine on the K^+-evoked release of [^{14}C]-dopamine (fractional release × 100/min) from tissue slices of nucleus accumbens is related (%) to parallel control incubations carried out in 34 mM K^+ in the absence of oxotremorine. Tissue slices derived from the same nucleus accumbens were preincubated together and divided into two and used for control and test conditions respectively. Values are means for the number of experiments indicated (*n*); vertical lines show s.e.means. The values of fractional release/min derived from the same nucleus accumbens for control and test conditions were compared using a paired Student's *t* test. *Indicates that the presence of oxotremorine significantly enhanced release, $P < 0.02$. The mean control value (fractional release × 100/min) of K^+-evoked release of [^{14}C]-dopamine (no drug) was 3.41 ± 0.05/min for 104 experiments.

as a fractional rate coefficient, i.e. the efflux at each time period as a percentage of the total radioactivity remaining at that time (e.g. % tissue [^{3}H]-acetylcholine released/min). This is defined as the amount of radioactivity collected during a 2.4 min period divided by the arithmetic mean of the total radioactivity present in the tissue at the beginning and end of the same collection period and divided by the time of collection. The K^+-evoked release is expressed as a percentage of the resting release in the sample immediately preceding the K^+ pulse (e.g. % increase in release of [^{3}H]-acetylcholine due to K^+).

Effect of oxotremorine on dopamine uptake

Dopamine uptake into tissue slices and homogenates of nucleus accumbens was measured in the same incubation medium as used for the release experiments. Tissue slices or unfractionated homogenates were preincubated (0.5 ml) for 30 min at 37°C in Krebs-bicarbonate medium containing glucose (10 mM), ascorbate (2.3 mM) and pargyline (1 mM). [^{14}C]-dopamine (56 mCi/mmol) was then added to give a final concentration of 0.7×10^{-7} M dopamine. Incubation was terminated after 5 min with unlabelled medium (3.0 ml) and the samples were immediately centrifuged. The pellet was washed 3 times with isotope-free incubation medium. The final pellet was digested with NaOH, mixed with Aquasol 2 and the radioactivity in the samples measured in a liquid scintillation spectrometer. The uptake into tissue slices was related to the wet weight of each tissue slice and uptake into homogenates was related to the protein content determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

Materials

All materials used were of Analar quality. Radioisotopes were obtained from The Radiochemical Centre, Amersham, Bucks. Oxotremorine and acetylcholine perchlorate were obtained from Sigma.

Results

Effect of oxotremorine on the release of [^{14}C]-dopamine from tissue slices of nucleus accumbens

Oxotremorine had little effect on the resting release of [^{14}C]-dopamine. However, the K^+-evoked release of [^{14}C]-dopamine was significantly enhanced by oxotremorine at concentrations of 1.6×10^{-7} M and above (Figure 1). The increase in release due to oxotremorine was 30% at 2×10^{-7} M oxotremorine and this increase remained constant up to a concentration of 10^{-4} M. The EC_{50} value for this stimulation

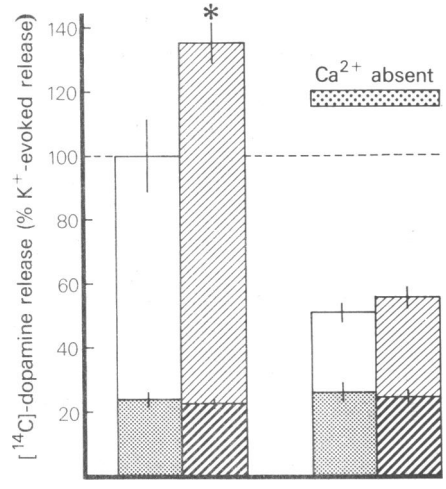

Figure 2 The effect of oxotremorine, in the presence and absence of calcium, on the K^+-evoked release of [^{14}C]-dopamine from tissue slices of nucleus accumbens is related to parallel control incubations carried out in 34 mM K^+ in the absence of oxotremorine (%). The medium containing no calcium contained $MgCl_2$ (2.5 mM) to maintain isotonicity. Values are means for 8 experiments; vertical lines show s.e.mean. *Indicates that oxotremorine significantly enhanced the K^+-evoked release of [^{14}C]-dopamine, $P < 0.01$. The mean control value (fractional release × 100/min) of K^+-evoked release of [^{14}C]-dopamine was 3.85 ± 0.47/min (8). Control incubation (stippled columns) superimposed on 34 mM K^+ incubation (open columns). Oxotremorine 3×10^{-4} M incubation (heavily hatched columns) superimposed on oxotremorine plus 34 mM K^+ incubation (lightly hatched columns.

was estimated to be 1.5×10^{-7} M. At concentrations of oxotremorine above 10^{-4} M, a further increase in the K^+-evoked release of [^{14}C]-dopamine was also seen. The effect of oxotremorine was not studied above 10^{-3} M. The enhancement of the K^+-evoked release of [^{14}C]-dopamine increased up to 50% at 10^{-3} M oxotremorine (Figure 1).

When $CaCl_2$ was replaced in the incubation medium with $MgCl_2$, the K^+-evoked release of dopamine was reduced by 66% (Figure 2). Oxotremorine up to a concentration of 3×10^{-4} M did not significantly increase the release of [^{14}C]-dopamine in the medium lacking Ca^{2+}. The K^+-evoked release of [^{14}C]-dopamine in the presence of oxotremorine was reduced by 72% in the calcium-free medium. These results indicated that calcium was necessary for both the K^+-evoked release and the enhancement of this effect by oxotremorine. Atropine (3×10^{-4} M) significantly antagonized the action of oxotremorine (Figure 3) but mecamylamine (10^{-4} M) was without effect.

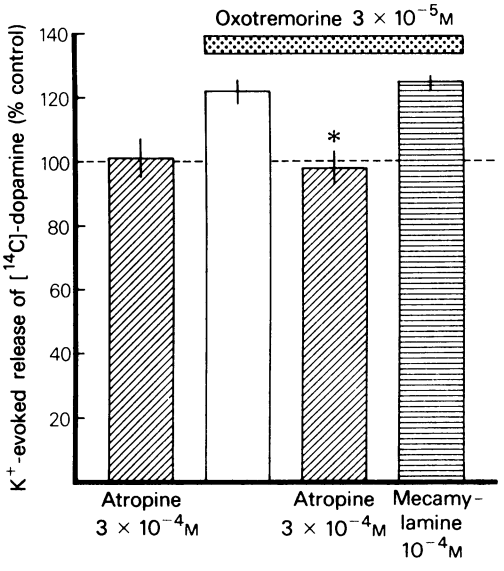

Figure 3 The effect of oxotremorine, in the presence and absence of atropine (3×10^{-4} M) and mecamylamine (10^{-4} M), on the K^+-evoked release of [^{14}C]-dopamine from tissue slices of nucleus accumbens is related to parallel control incubations carried out in 34 mM K^+ in the absence of drugs (%). Values are means for 5 experiments, vertical lines show s.e.mean. *Indicates that atropine significantly reduced the effect of oxotremorine, $P < 0.05$. Further details as for Figure 1.

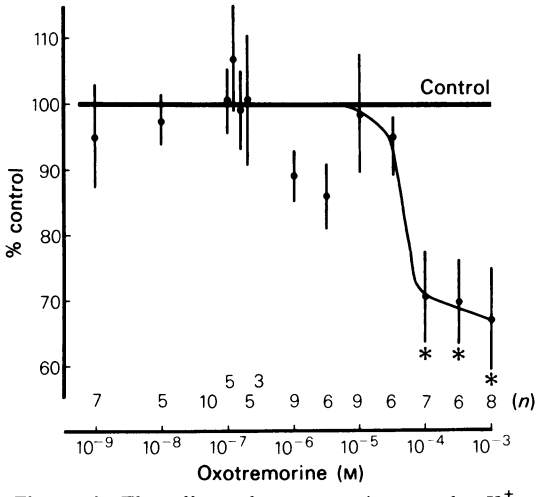

Figure 4 The effect of oxotremorine on the K^+-evoked release of [^{3}H]-acetylcholine (fractional release × 100/min) from tissue slices of nucleus accumbens is related to the release measured in parallel incubations (%) carried out in 34 mM K^+ in the absence of oxotremorine. Tissue slices derived from the same nucleus accumbens were preincubated together, divided and used for both control and test conditions. Values are means for the number of experiments indicated (n), vertical lines show s.e.mean. *Indicates that the values are significantly decreased by the presence of oxotremorine, $P < 0.02$. The mean control value of K^+-evoked release (fractional release × 100/min) of [^{3}H]-acetylcholine (no drug) was 2.9 ± 0.05/min for 78 experiments. Further details as for Figure 1.

Effect of oxotremorine on the release of [^{3}H]-acetylcholine

Oxotremorine did not affect the release of [^{3}H]-acetylcholine up to 10^{-5} M, which covered most of the range of concentrations causing the initial enhancement of the K^+-evoked release of [^{14}C]-dopamine (Figure 4). However, at 10^{-4} M oxotremorine, a significant inhibition (30%) of the K^+-evoked release of [^{3}H]-acetylcholine was seen. The second phase of stimulation of [^{14}C]-dopamine release was not evident at this concentration of oxotremorine. The IC_{50} for this inhibitory action of oxotremorine was estimated to be 4.3×10^{-5} M.

Effect of acetylcholine on [^{14}C]-dopamine and [^{3}H]-acetylcholine release from nucleus accumbens

Acetylcholine, 8.2×10^{-4} M in the presence of physostigmine (10^{-4} M) produced similar effects to the higher concentrations of oxotremorine, e.g. 10^{-4} M. Thus, acetylcholine significantly enhanced the K^+-evoked release of [^{14}C]-dopamine (50%) and also decreased the release of [^{3}H]-acetylcholine (30%) from tissue slices of nucleus accumbens (Figure 5). The magnitude of these effects on the K^+-evoked release of [^{14}C]-dopamine was similar and in the same direction as those obtained with oxotremorine, although a superfusion method was used for measuring the effect of acetylcholine compared to a 5 min incubation period for the latter. Using the superfusion method, it was possible to determine whether the effect of acetylcholine was reversible by applying a third pulse of K^+ in the absence of acetylcholine. The K^+-evoked release of [^{14}C]-dopamine in the third K^+ pulse was similar to the original response (first pulse), whereas the release of [^{3}H]-acetylcholine had only partially returned to the original response.

Effect of oxotremorine on dopamine uptake

In order to test whether the stimulatory effect of oxotremorine on dopamine release was partially due to an inhibition of dopamine uptake, the effect of oxotremorine on dopamine uptake into tissue slices and homogenates of nucleus accumbens was measured in the same incubation medium as used for the release experiments. Oxotremorine did not affect the uptake of [^{14}C]-dopamine (0.7×10^{-7} M) into tissue

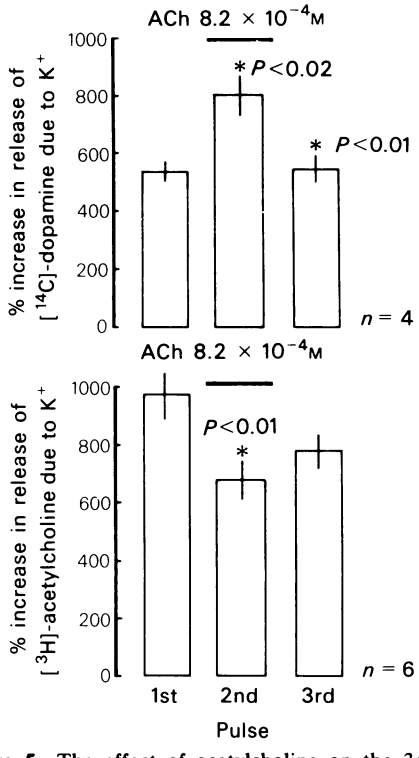

Figure 5 The effect of acetylcholine on the 34 mM K^+-evoked release of [^{14}C]-dopamine and [^{3}H]-acetylcholine from continuously superfused tissue slices of nucleus accumbens. Three pulses of K^+ were applied (4.8 min); acetylcholine was only present in the second pulse. The K^+-evoked release (fractional release/min) is expressed as a percentage of the resting release in the sample immediately preceeding the K^+-pulse (% increase in release due to K^+). Values are means for n experiments as indicated; vertical lines show s.e.mean. Further details described in the Methods.*Indicates that release is significantly different from that during previous K^+ pulse.

slices ($n = 5$), uptake of dopamine was 0.51 ± 0.04 (mean ± s.e.mean) pmol $5\,min^{-1}\,mg^{-1}$ wet wt in control conditions compared to 0.54 ± 0.04 in the presence of 10^{-6} M oxotremorine and 0.56 ± 0.05 in the presence of 3×10^{-7} M oxotremorine. Similarly, oxotremorine had no effect on an unfractionated homogenate of nucleus accumbens, where the rate of uptake was 34.2 ± 2.0 pmol $5\,min^{-1}\,mg^{-1}$ protein ($n = 5$) under control conditions and 37.3 ± 1.1 pmol $5\,min^{-1}\,mg^{-1}$ protein ($n = 6$) in the presence of oxotremorine (10^{-6} M). Although optimal rates of dopamine uptake would not be obtained using tissue slices and unfractionated homogenates these results clearly showed that under the experimental conditions used, where oxotremorine enhances dopamine release, it has no action on dopamine uptake.

Discussion

Modulation of dopamine release by cholinoceptor agonists

Both oxotremorine and acetylcholine enhanced the K^+-evoked release of dopamine. From the studies on oxotremorine, this effect is seen to be blocked by the muscarinic antagonist, atropine and dependent on the presence of calcium. The stimulation of dopamine release was maximal at 2×10^{-7} M oxotremorine. At considerably higher concentrations, e.g. 10^{-3} M, a further significant increase in release of dopamine was promoted by oxotremorine.

Modulation of acetylcholine release

Oxotremorine also had an effect on acetylcholine release, reducing the K^+-evoked release of [^{3}H]-acetylcholine. The IC_{50} for this inhibition was approximately 4.3×10^{-5} M. This action of oxotremorine contrasted with those seen on dopamine release, since it was inhibitory rather than excitatory and also, the half maximal effect occurred at concentrations more than one order of magnitude apart. It seems likely that the inhibition of acetylcholine release by a cholinoceptor agonist serves as an autoregulatory mechanism to suppress transmitter release from the nerve terminals when it has built up to a critical level in the synapse. A similar action of cholinoceptor agonists on acetylcholine release has also been demonstrated in cerebral cortex (Szerb & Somogyi, 1973).

Presynaptic muscarinic receptors on dopamine terminals

A direct modulation of dopamine metabolism and release in the nucleus accumbens by a transmitter or drug is likely to be mediated by a presynaptic receptor, for example, the regulation of dopamine synthesis by apomorphine in the striatum (Christiansen & Squires, 1974). The possibility that cholinoceptors may be present on dopamine terminals emerges from these release studies in nucleus accumbens presented here and similar studies on other dopaminergically innervated regions such as corpus striatum (Giorguieff, Le Floc'h, Westfall, Glowinski & Beeson, 1977; de Belleroche & Bradford, 1978) and is supported by studies on muscarinic receptor populations. Thus, 6-hydroxydopamine treatment which destroys dopamine terminals also depletes the levels of muscarinic receptors in corpus striatum (Kato, Carson, Kemel, Glowinski & Giorguieff, 1977; de Belleroche, Luqmani & Bradford, 1978) and nucleus accumbens (de Belleroche *et al.*, 1981b). A detailed examination of the class of muscarinic agonist bind-

ing site depleted in the nucleus accumbens by 6-hydroxydopamine lesion of dopamine containing cells of the ventral tegmental area indicated that there is a selective loss of the highest affinity binding site, known as the 'super high' affinity binding site (de Belleroche *et al.*, 1981b). Thus, presynaptic muscarinic receptors capable of being depleted by 6-hydroxydopamine are likely to be mediators of the action of oxotremorine and acetylcholine on dopamine release from nucleus accumbens.

Functional role of interaction between cholinergic and dopaminergic systems in the nucleus accumbens

The dopaminergic innervation of nucleus accumbens plays a key role in modulating locomotor activity as shown by the action of dopamine and dopamine agonists applied directly into the nucleus accumbens of rats to increase locomotor activity and exploration (e.g. Pijnenburg & Van Rossum, 1973; Costall & Naylor, 1975). This effect contrasts with that obtained from injection of dopamine into the caudate nucleus also innervated by a dopamine projection from the midbrain which causes gnawing, biting and licking. Other behavioural measures such as hoarding and maternal behaviour are lost on 6-hydroxydopamine lesion of the ventral tegmental area (Le Moal, Galey & Cardo, 1975; Galey, Simon & Le Moal, 1977) and are therefore dependent on an intact dopaminergic innervation of the nucleus accumbens. The present results indicate that the presynaptic action of cholinoceptor agonists modulate dopaminergic activity. These findings are consistent with the action of intra-accumbens injections of oxotremorine and carbachol to enhance dopamine-induced hyperactivity (de Belleroche *et al.*, 1981a; Jones *et al.*, 1981). This stimulatory action occurs within the first hour following injection of the agonist and contrasts with the delayed action of cholinergic agents, e.g. arecoline and physostigmine, which after 1–2 h cause a marked inhibition of locomotor activity (Costall *et al.*, 1980).

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