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A PHARMACOLOGICAL INVESTIGATION OF THE ELECTRICALLY EVOKED CONVULSIVE ACTIVITY INDUCED BY ADMINISTRATION OF CATECHOL IN THE ANAESTHETIZED RAT

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1 The response evoked by electrical stimulation at the wrist has been recorded from muscles of the forelimb of anaesthetized rats induced to convulse by administration of catechol.

2 This response can be divided into three temporally distinct components, the characteristics of which have been described.

3 The probability of occurrence of the two early components of the response has been measured before and after administration of various drugs. The results show that the first component is not affected by cholinoceptor or adrenoceptor blocking drugs or anticholinesterase agents. The probability of occurrence of the second component is significantly reduced by cholinoceptor blocking drugs and increased by physostigmine.

4 The implications of these results in explaining the convulsant actions of catechol are discussed.

Introduction

Administration of catechol to anaesthetized or unanaesthetized animals produces a wide spectrum of motor activity as the dose of the drug is increased. This ranges from tremor, to sensory myoclonic effects to spontaneous convulsions. These motor effects together with the relatively rapid degradation and excretion of catechol may make it a suitable model for the study of epileptogenesis.

A previous paper (Angel, Clarke & Dewhurst, 1977) showed that spontaneous catechol convulsions, occurring in the absence of sensory stimuli, were attenuated by cholinoceptor blocking drugs and exacerbated by anticholinesterase agents. This study has been extended by investigation of the pharmacology of the central pathways involved in sensory evoked muscle jerks induced by administration of catechol. The evoked jerks, which are totally absent in the deeply anaesthetized, non-catechol treated animal have been studied in some detail (Angel & Lemon, 1973a, b; 1975) and have been shown to consist characteristically of three temporally distinct components. Each of these components arises from an action of catechol on different central pathways and in the rat the pathways for the two shorter latency components have been described (Angel & Lemon, 1973a, b; 1975). The present study investigates the pharmacology of the pathways involved in the two early components of evoked jerks, which may afford some insight into both the mechanism and site of action of catechol. A preliminary account of this work has been published (Angel & Dewhurst, 1975).

Methods

Female albino rats (Sheffield strain) in the weight range 190 to 210 g were used in all experiments. Animals were anaesthetized with urethane (1.25 g/kg i.p.) such that reflex withdrawal of the hind-limb to a strong pinch of the paw was just abolished. The trachea was intubated and polythene cannulae inserted into the jugular vein and carotid artery. The latter was filled with heparinized saline (1000 units/ml) and attached via a three-way tap to a pressure transducer (Bell and Howell Ltd Type 4-327 L221), in order to monitor the animal's blood pressure. Body temperature was maintained at 37°C by means of an overhead heating lamp and monitored by means of a rectal thermometer.

Recording electrodes consisting of two fine hypodermic syringe needles were positioned in the body of the muscle under study (usually Flexor carpi), and rigidly clamped to prevent movement. The forelimb

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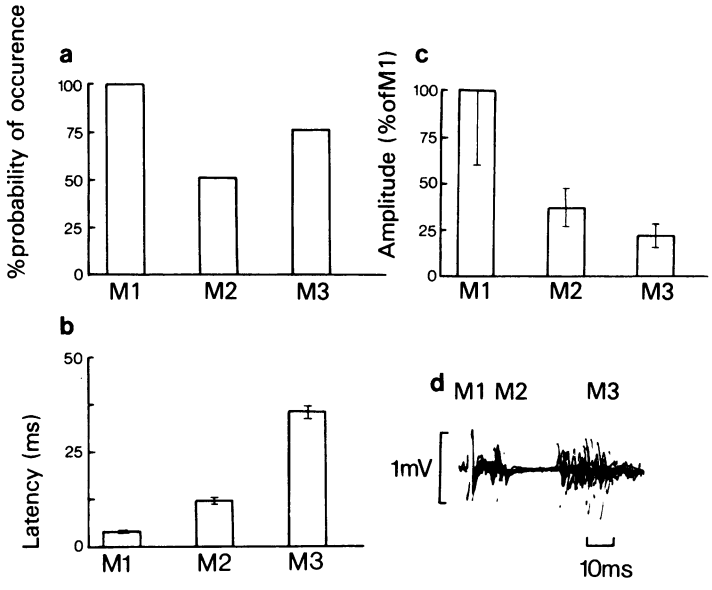

Figure 1 The essential characteristics of the sensory evoked muscle response induced by catechol administration. (a) The percentage probability of occurrence of each of the three components M1, M2 and M3. (b) Latency distribution. (c) Relative amplitudes of each component. (d) Photographic record showing 20 superimposed responses recorded from Flexor carpi to consecutive electrical stimuli applied at the wrist. Results are the means of 10 experiments; vertical bars show s.d.

was outstretched and fixed in position by means of pins inserted through skin folds, such that movement of the forelimb was minimized. Recordings were monitored on a cathode-ray oscilloscope and simultaneously recorded on magnetic tape. (Akai GXC 36D cassette recorder).

Muscle responses were evoked by electrical stimuli applied to the forepaw by means of lint strips soaked in 3 M NaCl, tied loosely around a digit and the wrist. This mode of stimulation has been shown to activate mainly cutaneous nerves (Angel & Brown, 1967). Stimuli were always of 50 μs duration, delivered at a frequency of 10/min, at a voltage adjusted to be just supra-threshold for the first component of the evoked muscle response (EMR) (usually of the order of 7 to 15 V).

The experiments can be divided into two types; those in which catechol was infused intravenously (3.7 mg kg^{-1} min^{-1}) for the duration of the experiment and those in which catechol was administered as a single intraperitoneal injection (60 mg/kg). In the infusion experiments the frequency of occurrence of the two early components of the evoked muscle response (M1 and M2) was measured before and after administration of the test drug. The experiment was divided for convenience into 2 min periods, each period containing the responses to 20 consecutive, electrical stimuli. The measured frequency of occurrence of both M1 and M2 per number of stimuli applied was then expressed as a probability of occurrence such that if each component occurred 20 times per 2 min period, the probability of occurrence was taken as unity. After a suitable control period (usually 12 min), the test drug was administered (i.p.) and its effects on the frequency of occurrence of each component recorded for approximately 30 to 40 min after administration. These effects were quantified by plotting the probability of occurrence for each component against time, after administration of the test drug and subtracting the area under this curve from the area under the control curve obtained by extrapolating the mean probability of occurrence of each component in the measured control period. The difference in areas was expressed in arbitrary units, as shown in Table 1. Thus a drug which decreases the probability of occurrence of one component will have a positive difference (control-test) while one which increases will have a negative difference. Statistical significance of difference between control and test periods was assessed by the paired t test.

In the second type of experiment, catechol was administered as two single intraperitoneal injections (60 mg/kg) given exactly 60 min apart. The resulting convulsive activity after each injection of catechol was recorded for 20 min, and again for convenience each of these 20 min periods was divided into shorter (1 min) periods. The probability of occurrence of M1 and M2 was measured as for the infusion experi-

ments, and plotted against time after injection of catechol. Test drugs were administered during the interval between the two injections of catechol such that the peak effect of the drug coincided approximately with the peak of the convulsive response to the second injection of catechol. We thus had in a single animal a control and post-test drug response such that the probability of occurrence of M1 and M2 could be compared before and after drug treatment. Control experiments were also performed in which an identical volume of 0.9% w/v NaCl solution (saline) was administered in place of the test drug.

In both types of experiment the dosage of catechol used was found sufficient to produce convulsions in all animals studied. All drugs were dissolved in saline, and all test-drugs were given intraperitoneally in a volume of 1 ml.

Results

The evoked muscle response induced by administration of catechol was found to consist characteristically of three temporally distinct components (Figure 1). The first (M1) was of short latency (4.21 ± 0.20 s.d. ms). The second (M2) of mean latency (12.20 ± s.d. 0.83 ms) and the third (M3) of longer latency (35.73 ± s.d. 1.55 ms). In an anlysis of ten experiments the respective amplitudes of the three components, expressing the amplitude of M1 as 100% were 37.2% for M2 and 23.6% for M3. The peak probability of occurrence per applied stimulus was 1.0, 0.51 and 0.76 for M1, M2 and M3 respectively. It was also observed that if M1 was of large amplitude, then M2 and M3 occurred less frequently and were generally of smaller amplitude and longer latency, and conversely if M1 was of low amplitude then M2 and M3 were more evident. Accordingly the stimulus voltage was critical and was carefully adjusted at the start of each experiment so that it was just supra-threshold for M1.

In experiments in which catechol was administered as a single intraperitoneal injection, M1 was found to reach a peak probability of occurrence at between 1 to 3 min after injection, and then to decline gradually to almost base-line levels after approximately 14 min. M2 appeared at about the same time as M1, but reached its peak probability of occurrence later than M1 (5 min). The probability of occurrence of M2 had also declined to zero 14 min after injection. M3 showed a similar time course to M1, reaching its peak probability of occurrence at between 1 to 3 min after injection but was totally absent 12 min after injection.

Effects of drugs

The effects of certain test-drugs on the probability of occurrence of M1 and M2 in catechol-treated rats have for convenience been divided into two groups.

Infusion experiments None of the drugs tested had any facilitatory effect, as measured by the amplitude, or inhibitory effect (as measured by a decrease in probability of occurrence) on the M1 component.

The effect of drugs on the probability of occurrence of the M2 component is shown in Table 1. The results are expressed as the mean difference between control and test response, as described in the methods. Examples of drugs which cause an increase (physostigmine) a decrease (atropine) and no change (neostigmine) in the probability of occurrence of M2 are shown in Figure 2.

Table 1 The effects of various drugs on the percentage frequency of occurrence of the second (M2) component of the evoked muscle response induced by administration of catechol

Drug	*Dose* (mg/kg)	*M2* Mean difference between control and test response, arb. units ± s.e. (*n*)	P
Atropine sulphate	40	74.3 ± 18.9 (7)	<0.01*
Hyoscine HBr	40	25.4 ± 12.3 (4)	<0.1
Atropine $MeNO_3$	20	−26 ± 17.4 (5)	<0.3
Mecamylamine HCl	2.5	21.7 ± 3.7 (4)	<0.001*
Hexamethonium Br	10	10.9 ± 9.5 (5)	<0.4
Physostigmine	0.04	−19.5 ± 7 (7)	<0.02*
Neostigmine Br	0.025	−3.8 ± 23.8 (5)	>0.8
(±)-Propranolol HCl	5	41.4 ± 13.1 (4)	<0.1
Phentolamine mesylate	10	1.8 ± 25.7 (4)	>0.9

* Significantly different from paired *t* test at 5% level.
n = number of experiments (see Methods for explanation).

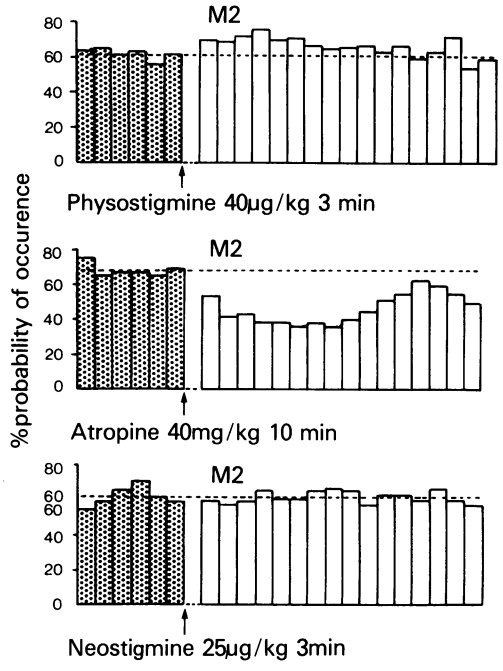

Figure 2 Effects of physostigmine (a), atropine (b) and neostigmine (c) on the percentage probability of occurrence of the M2 component of the evoked muscle response. Catechol was infused intravenously for the duration of the experiment and each drug was administered as indicated by the arrow. Each column represents a 2 min recording period. The horizontal dashed line represents the mean percentage probability of occurrence of the M2 component in the control period (stippled columns). Results are the means of 7 (atropine, physostigmine) and 5 (neostigmine) experiments.

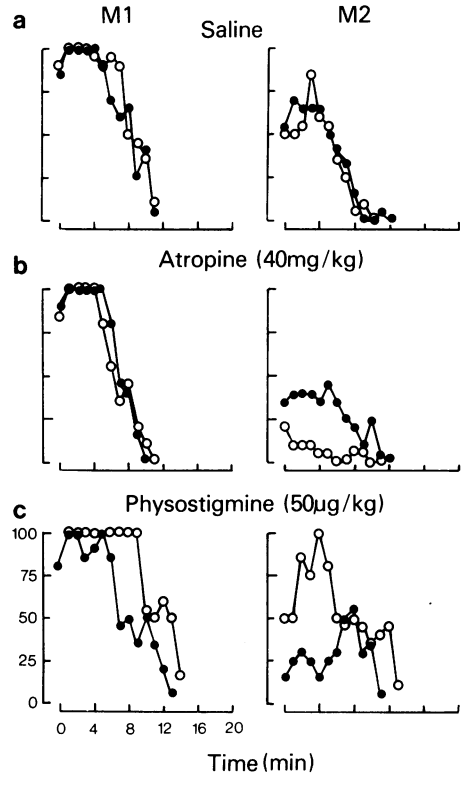

Figure 3 Typical single experiments to show the effects of the drugs atropine (b) and physostigmine (c) on the percentage probability of occurrence of the M1 (left) and M2 (right) components of the evoked muscle response induced by catechol administration to the anaesthetized rat. Catechol was administered as two single injections exactly 60 min apart, and the 20 min evoked muscle activity recorded after each injection. Each graph shows the percentage probability of occurrence of M1 and M2 in the first 20 min (C1 ●) and in the second (C2 ○) superimposed. The test drugs were administered in the interval between catechol injections such that their effects on the probability of occurrence of M1 and M2 in C2 could be studied. Thus C1 represents the control period and C2 the post-test drug period for each animal. For comparison the control experiment of substituting saline injection for the test drug is also shown (a).

As Table 1 shows, only atropine and mecamylamine significantly decreased the probability of occurrence of M2 while only physostigmine increased it. The onset of the effects of atropine and mecamylamine were similar, beginning 12 min after injection of the test drug, although the effects with atropine were more prolonged, lasting 20 min as compared to 12 min for mecamylamine. Physostigmine increased the probability of occurrence of M2 within 3 min of injection, the peak effect occurring 10 min after injection and lasting about 35 min.

Single injection experiments Typical effects of two drugs atropine (40 mg/kg) and physostigmine (50 μg/kg) are shown in Figure 3, compared to the control experiment of injecting saline. In the control experiments, the probability of occurrence of both M1 and M2 showed a similar time course after injection of catechol at 0 and 60 min. The probability of occurrence of both M1 and M2 reached a peak quickly after administration of catechol; within 2 min for M1, and from 2 to 5 min after injection for M2. In all cases the probability of occurrence decreased rapidly from the peak, and was back to zero within 20 min of catechol administration. The probability versus time plots for both M1 and M2 after each injection of catechol superimposed almost exactly in control

experiments, though the probability of occurrence was always slightly higher after the second injection of catechol than after the first. Atropine had little effect on the probability of occurrence of M1 though it was reduced slightly, but did have a marked effect on that of M2 (Figure 3). Physostigmine prolonged the action of catechol, in that of the maximum probability of occurrence of M1 was maintained for longer and also increased the probability of occurrence of M2. This latter effect was seen consistently in all experiments but was masked in averaging experimental results due to considerable variations in both the control probability of occurrence of M2 from experiment to experiment, and variations in the latency of the increased probability, seen after physostigmine. Neither of the drugs significantly affected the amplitude of M1.

Discussion

Administration of catechol to anaesthetized rats induces an excitatory state during which muscular jerks can be evoked by appropriate sensory stimulation. These jerks are totally absent in the deeply anaesthetized, non-catechol treated animal and records show the muscles to be electrically quiescent. The results presented in this paper show the effects of certain drugs on muscle activity (EMR) recorded from a forelimb flexor muscle of the anaesthetized, catechol-treated rat to electrical stimulation at the wrist.

The discharge probability, latency and amplitude characteristics of the EMR during continuous intravenous infusion of catechol are in good agreement with the findings of Angel & Lemon (1973b), who used a different forearm muscle and administered catechol as a single intraperitoneal injection. The major difference is the much higher discharge probability of the third component of the evoked muscle response (0.76 compared to 0.27).

It appears that catechol exerts its effects either directly or indirectly on at least three distinct central pathways, giving rise to the three components of the EMR. The first component has been shown to be a purely spinal reflex (Angel & Lemon, 1973 b), while there is much evidence that the second component is dependent on activation of a long-loop reflex from the periphery, through the cortex and thence to the muscle. (Angel & Lemon 1973 b; 1975). The longer-latency third component of the EMR is the least understood but it has been suggested that it corresponds to a spino-bulbo spinal reflex, dependent on the integrity of the cerebellum (Lemon, 1971).

The effects of various drugs on the two early components of the EMR showed that the first component was unaffected by drugs which modify adrenergic and cholinergic transmission while the discharge probability of the second component was decreased by cholinoceptor blocking agents and increased by the centrally acting anticholinesterase, physostigmine. This indicates that transmission through the spinal reflex pathway is not dependent on or modified by adrenergic or cholinergic neurones, but suggests that the cortical reflex pathway contains or is modified by cholinergic neurones. The similar action of the cholinoceptor-blocking agents, atropine and mecamylamine, suggest that if catechol is exerting its effects via a cholinergic system, then this effect is rather non-specific, involving both muscarinic and nicotinic acetylcholine receptors. The lack of effect of the peripherally acting cholinoceptor blockers, atropine methyl nitrate and hexamethonium, indicates that catechol is primarily acting at central sites. The increased probability of discharge of M2 after physostigmine may indicate that catechol is releasing acetylcholine at certain central synapses, and that the released acetylcholine is then exerting the primary excitatory effect, these effects being prolonged after cholinesterase inhibition. The lack of effect of neostigmine, which does not cross the blood-brain barrier seems to rule out a possible potentiation of acetylcholine effects at the neuromuscular junction, and is supported by the observation that there were no signs of iterative muscle discharge in the spinal component of the EMR.

Some of the drugs used also had pronounced effects on blood pressure but it would appear that these effects are unrelated to any effects on the evoked muscle response. For example, hexamethonium and phentolamine produced an equivalent or greater fall in blood pressure than atropine but had no effect on the cortical component of the EMR.

The cortical reflex pathway of the M2 component would appear to involve sensory afferents ascending to the cortex in the dorsal column medial lemniscal system (Angel, 1969) where they synapse with cortical pyramidal tract neurones. Catechol has been shown to exert its chief effects on thalomo-cortical transmission by increasing the modulatory effects of thalamic reticular cells of the ventro-basal thalamus (Angel, 1969). There is also a large body of both histological, (Shute & Lewis, 1963; 1967) and pharmacological evidence (Krnjević, 1967; Bradley & Dray, 1972) to suggest that acetylcholine is a possible excitatory transmitter involved in reticulo-cortical arousal, and evidence has been put forward of a specific cholinergic pathway between thalamus and cortex (Phillis, 1971). The ratio of muscarinic cholinoceptors to nicotinic has also been shown to be relatively high in this brain area (McCance, Phillis & Westerman, 1968; Hiley & Burgen, 1974).

The results with drugs affecting cholinergic transmission are thus in agreement with the physiological sites of action of catechol, and the evidence

that acetylcholine is a putative excitatory transmitter at these sites. The greater effectiveness of muscarinic blocking drugs as compared to nicotinic blocking drugs may be a reflection of the high muscarinic to nicotinic receptor ratio in the thalamus and thalamic reticular formation.

There is thus a close similarity in terms of their sensitivity to drugs which modify cholinergic transmission, between the cortical component of the EMR in the catechol-treated, anaesthetized rat and spontaneous catechol convulsions in mice. This may indicate that the pathways involved in giving rise to the cortical component may also be important in catechol-induced spontaneous convulsions.

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