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A PHARMACOLOGICAL STUDY OF THE SPONTANEOUS CONVULSIVE ACTIVITY INDUCED BY 1,2-DIHYDROXYBENZENE (CATECHOL) IN THE ANAESTHETIZED MOUSE

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1 The convulsive activity induced by catechol has been examined in anaesthetized mice either by determining the CD_{50} for the convulsions in drug-treated and control animals, or by studying the effects of various drugs on the total whole body activity.

2 The results indicate that catecholamines play no part in the mechanism of action of catechol. Drugs which alter cerebral catecholamine levels had no effect on the convulsions, nor did the α- and β-adrenoceptor blocking drugs.

3 5-Hydroxytryptamine (5-HT) could possibly be important, though results with drugs which either change brain 5-HT levels, or block 5-HT receptors were inconsistent.

4 γ-Aminobutyric acid also appears not to be involved in the mechanism of action of catechol.

5 The results strongly suggest that catechol primarily activates a central cholinergic system, in that muscarinic and nicotinic receptor blocking drugs inhibit, and anticholinesterases potentiate the convulsions.

Introduction

The choice of catechol as the chemical to use in a model of epileptogenesis was for a variety of reasons: (a) depending upon the dose administered the chemical gives tremor, sensory evoked muscular jerks or spontaneous convulsions; (b) it is rapidly removed by *O*-methylation to produce inactive guaiacol; (c) its LD_{50} and CD_{50} are in the ratio of 4:1; (d) in the un-anaesthetized animal it gives a 4–5 min convulsive period followed by 40–50 min post-ictal depression after which the animal is behaviourally indistinguishable from normal, and (e) it produces convulsions even in the surgically anaesthetized animal. Three main mechanisms have been proposed for the convulsive action of catechol.

First Bacq, Gosselin, Dresse & Renson (1959) proposed that it was as a result of inhibition of catechol-*O*-methyl transferase (COMT) and a sensitization to endogenous cerebral noradrenaline. This followed from the observations that catechol could sensitize smooth muscle to both electrical stimulation of adrenergic nerve fibres and to the injection of adrenaline (Bacq, 1936). However, Matsumoto & Nishi (1963) and Rogers, Angel & Butterfield (1968) found no changes in cerebral amine levels during catechol-induced convulsions, and Angel & Rogers (1968) showed that more potent COMT inhibitors than catechol were either ineffective or were much less active as convulsant agents.

Secondly, Hochstein & Cohen (1960) demonstrated that catechol inhibits glycolysis in brain cell homogenates and proposed that a fall in cerebral adenosine triphosphate (ATP) could give rise to 'anoxic-like' convulsions. Angel, Lemon, Rogers & Banks (1969) showed that 1,2,3-trihydroxybenzene (pyrogallol) gave a similar reduction in cerebral ATP to that of catechol but did not produce any convulsive activity, and further that animals respiring 95% O_2 and 5% CO_2 showed no fall in cerebral ATP levels after catechol but still convulsed.

Thirdly, the action of catechol at the neuromuscular junction where it increases the amount of acetylcholine liberated per nerve impulse (Mogey & Young, 1949; Blaber & Gallagher, 1971) suggests a possible central cholinergic mechanism for its convulsant activity. Preliminary experiments in which physostigmine was found to potentiate and atropine to block partially the convulsions (Angel, 1969) led some support to this view.

The present study is an attempt to elucidate further the mechanism of action of catechol by using drugs which modify the synthesis, breakdown or action of various putative neurotransmitters. A preliminary account of the work has been published (Angel & Dewhurst, 1975).

Methods

Spontaneous convulsions were studied in mice (Sheffield strain) of either sex in the weight range 16–25 grams. Convulsive activity was measured by one of two methods: (i) total body activity and (ii) convulsive dose$_{50}$ (CD_{50}) estimations.

Total body activity

Groups of four mice were anaesthetized with ethyl carbamate (2 g/kg intraperitoneally). Total activity was then measured by placing the animals supine in a plastic container, suspended by a stiff wire from a strain gauge. Any movement of the animals within the beaker resulted in a deformatory stress being applied to the gauge; the latter being incorporated in a bridge circuit. The bridge output was amplified, half-wave rectified and integrated over 30 s intervals, and the integrator output led into a single channel pen-recorder, for a permanent record. The animal's body temperature was maintained at 36–37°C by enclosing the apparatus in a polystyrene box, in the bottom of which was a copper radiator, through which water at 40°C was circulated and by an overhead heating lamp.

Early in the study it was found that the response of different groups of mice to the same dose of catechol (80 mg/kg i.p.) was extremely variable both temporally and in intensity. The extremely large variation for 80 groups of mice (from 90–545 arbitrary units of activity) rendered it impossible to study the effects of drugs simply by comparing the responses of control groups with those of drug-treated groups. However, it was found that the responses of a single group of mice to two successive doses of catechol (80 mg/kg i.p.) were similar and that the ratio of total activity to the second dose (C2) over that to the first (C1) was constant provided that the time between doses was not less than 40 min and that the animal's body temperature was maintained at 37°C. Accordingly the following experimental regime was adopted. Basal activity was recorded for 15 min before each injection of catechol (80 mg/kg). The activity in the 20 min period after injection of the group was measured and corrected for basal activity to give a measure of the convulsive activity. The ratio of convulsive activity for the second versus the first dose was then determined, i.e. C2 : C1 and used as the index for comparing control and drug-treated groups of animals. All drugs were administered intraperitoneally dissolved in 0.9% w/v NaCl solution (saline) at suitable time intervals before the second injection of catechol such that their maximum effect coincided approximately with the peak of the second convulsive response. The drugs were made up so that the stated dose required an injection volume of 1.0 ml/100 g body weight. Control animals were treated with an equivalent volume of saline.

CD_{50} estimations

The effects of drugs which needed either chronic or long pre-treatment were evaluated by determining their effects on the catechol CD_{50} by the method of Weil (1952) on mice anaesthetized with ethyl carbamate (2 g/kg intraperitoneally). Groups of ten mice were used and estimations for control (saline pretreated with a schedule identical to that of the drug pretreatments) and experimental groups were performed simultaneously. Care was taken to keep the experimental environment quiet since the CD_{50} for auditory evoked jerks is less than that for the spontaneous convulsions (31.2 mg/kg and 40.7 mg/kg respectively).

Muscle spindle activity

In a few experiments anaesthetized (urethane, 1.25 g/kg) rats (Sheffield strain, female, weight range 190–210 g) were used to study the effects of physostigmine and atropine on the discharge of muscle spindle afferents originating in muscles of the hindlimb and recorded from split dorsal root filaments after laminectomy from T12–L6. The limb was firmly clamped and the muscles under study were fixed at, or near, body length. Conventional recording techniques were used.

Results

Convulsant activity

The CD_{50} for catechol was found to be 40.7 mg/kg and a dose of 80 mg/kg was chosen for the determination of the activity ratio to two successive doses because all animals showed some convulsive activity at this dose and it was sublethal. The peak resultant activity was seen 2.5 min after injection and the decay from this peak was found to be exponential with a half life of 4.9 minutes. Thus the definition of convulsive activity used to determine the activity ratios (i.e. the total activity, in arbitrary units, corrected for basal activity in the 20 min period following injection of the 4th animal of the group) takes into account both any change in peak activity or any prolongation of the effect of the second dose of catechol by drug action.

The results are presented in tabular form for CD_{50} (Table 1) and activity ratios (Table 2). For the activity ratios, statistical significance of difference between control and drug-treated groups was assessed by the Mann-Whitney U test. For the CD_{50} estimations, differences are considered as statistically significant if the $CD_{50} \pm 95\%$ confidence limits (Weill, 1952) do not overlap.

From Table 1 it can be seen that drugs which affect

gross levels of brain amines (monoamine oxidase (MAO) inhibitors, reserpine, L-DOPA, 6-hydroxydopamine) have no effect on the catechol CD_{50}. In contrast *p*-chlorophenylalanine (PCPA) significantly elevated the CD_{50} as did combined PCPA and 5-hydroxytryptophan (5-HTP) treatment. Atropine also elevated the CD_{50} from a control value of 43.2 mg/kg to 64.2 mg/kg, showing that the two methods of evaluating the effects of drugs on convulsive activity used, are comparable.

From Table 2 it is clear that drugs affecting cholinergic systems (nicotinic and muscarinic blocking drugs, anticholinesterases) either significantly inhibit (blocking drugs) or potentiate (anticholinesterases) the convulsions, with the exceptions of atropine (at low doses) and atropine methyl nitrate (at all doses). Oxotremorine too had no significant effect on the convulsions.

Of the other drugs used only propranolol, pyrogallol and L-tryptophan significantly affected the convulsions, propranolol significantly decreasing and L-tryphophan and pyrogallol significantly increasing the activity ratio.

Muscle spindle activity

Physostigmine. Fourteen spindle afferents were studied in 14 animals (9 from innervated, 5 from de-efferented muscles). Of the 14 only 5 showed a spontaneous discharge. None of the 9 afferents without resting discharge showed any activity after the intraperitoneal injection of physostigmine 40 μg/kg; neither were there any marked changes in discharge frequency of the spontaneously active afferents. The greatest change seen was from 6.7 to 8.5 impulses/s 10 min after physostigmine injection. From a large population (176) of spindle afferents a normal minute to minute variation of ±5 impulses/s has been found.

Atropine. Similar results were obtained with atropine (40 mg/kg); 8 afferents (from 8 animals) were studied. Of these 5 were from de-efferented and 3 from innervated muscles. Only 3 showed spontaneous activity of 18.0, 15.7 and 8.4 impulses/s before and 18.0, 14.9 and 11.5 impulses/s respectively after intraperitoneal injection of atropine. One muscle which was subjected to sinusoidal stretch (1 Hz, 500 μm p–p

Table 1 Effects of drugs needing long pre-treatment times on the catechol CD_{50}

Drug	*Dose (mg/kg)*	*Pretreatment conditions*	*CD_{50} (mg/kg)*	*95% CL*	*Sig.*
Pargyline HCl	50 (i.p.)	5 h	36.8	32.8–41.2	NS
Control	Saline	5 h	36.8	31.5–42.9	
Iproniazid PO_4	50 (i.p.)	5 h	42.8	32.7–56.2	NS
Control	Saline	5 h	38.7	30.3–49.4	
Reserpine	1 (i.p.)	15 h	34.3	28.1–41.9	NS
Control	Saline	15 h	36.8	31.5–42.9	
L-DOPA	200 (i.p.)	30 min	39.8	34.4–46.2	NS
Control	Saline	30 min	41.5	34.7–48.5	
Parachlorophenyl-alanine (PCPA)	150 (i.p.)	2 × daily 3 days, test on 4th day	63.4	56.5–71.8	B
Control	Saline	2 × daily 3 days, test on 4th day	43.2	36.4–51.3	
PCPA + 5-hydroxy-typtophan	150 (i.p.) 100 (i.p.)	As above 30–60 min before test	64.8	58.7–71.6	B
Control	Saline	As above 30–60 min before test	43.9	37.6–51.3	
6-Hydroxydopamine	80 μg in 10 μl i.c.v.	1 week	43.9	37.6–51.3	NS
Control	Saline	1 week	43.9	37.6–51.3	
Atropine SO_4	50 (i.p.)	15 min	64.2	43.3–95.1	B
Control	Saline	15 min	43.2	36.4–51.3	

CL = confidence limits; B = block; NS = not significant.

Table 2 Effects of drugs requiring short pretreatment times on the catechol convulsive activity ratio

Drug	*Dose (mg/kg)*	*Injection time before C2 (min)*	*No. of groups*	*Median activity ratio*	P	*Effect*
Saline	–	15	12	1.36	–	–
Pyrogallol	100	10	9	2.30	0.05	P
Phentolamine mesylate	5	15	8	1.27	–	–
(±)-Propranolol HCl	2	15	11	1.07	0.05	B
(+)-Propranolol HCl	5	15	8	0.98	0.01	B
Sotalol	5	15	10	1.42	–	–
L-Tryptophan	200	35	12	1.9	0.01	P
Methysergide bimaleate	2	25	8	1.39	–	–
Apomorphine HCl	20	25	10	1.18	–	–
(+)-Amphetamine SO_4	5	10	11	1.55	–	–
GABA	100	15	8	1.89	–	–
Amino-oxyacetic acid	25	65	8	1.37	–	–
Atropine SO_4	5	15	6	1.26	–	–
	10	15	8	0.86	0.05	B
	20	15	8	0.65	0.001	B
Hyoscine HBr	20	15	12	0.84	0.001	B
Atropine $MeNO_3$	5	15	7	1.46	–	–
	20	15	8	1.71	–	–
Mecamylamine HCl	2	15	10	1.08	0.01	B
	5	15	9	0.78	0.01	B
Pempidine tartrate	5	15	8	0.94	0.05	B
	10	15	11	0.86	0.001	B
Hexamethonium Br	2	15	10	0.90	0.05	B
	5	15	9	0.92	0.001	B
Dihydro-β-erythroidine	0.5	15	11	0.87	0.001	B
Physostigmine	0.002	10	9	3.40	0.001	P
Neostigmine Br	0.025	10	9	2.12	0.001	P
Oxotremorine	0.01	15	17	1.86	–	–

P = potentiation; B = block; – = not significantly different.

amplitude) showed little change in peak discharge frequency after atropine; 15.9 impulses/s before, 14.9 impulses/s after.

Discussion

Catecholamines

Gross levels of catecholamines can be altered, to different degrees by reserpine (Kirshner, 1962) and the MAO inhibitors (Zeller & Fouts, 1952) but with no effect on the catechol-induced convulsions. Similarly 6-hydroxydopamine, which selectively destroys adrenergic and dopaminergic neurones (Thoenen & Tranzer, 1973), and L-DOPA a precusor of dopamine (Blaschko, 1939), had no effects on the catechol CD_{50}. These results, while not ruling out a possible catecholaminergic link in the mechanism of action of catechol, do at least suggest that catechol does not affect presynaptic catecholaminergic mechanisms. The COMT inhibitor pyrogallol (Axelrod & Laroche, 1959) which increases cerebral catecholamine levels (Biscardi & Izquierdo, 1961) significantly potentiates the effect of catechol, chiefly by prolonging its action (catechol half-life after pyrogallol is 7.9 minutes). It is unlikely that this effect is due to an increase in brain catecholamines since MAO inhibitors are ineffective, and it can probably be accounted for by the fact that both catechol and pyrogallol compete as substrates for COMT (Axelrod & Laroche, 1959; Bacq *et al.*, 1959), the most probable enzyme for the elimination of injected catechol (Angel & Rogers, 1972). The lack of effect of drugs which modify brain amine levels is in agreement with the observations of White & Nash (1963) and Angel & Rogers (1968) who showed that neither reserpine nor chlorpromazine affected either the electrocortical desynchronizing or convulsive actions of catechol. It has also been shown (Angel, Rogers & Butterfield, 1968) that catechol has no effect on cerebral noradrenaline and dopamine concentrations.

Although the results discussed above rule out a presynaptic effect of catechol on aminergic neurones,

a post-synaptic effect is still possible, particularly since the catechol nucleus is present in both noradrenaline and dopamine. If catechol were in some way affecting postsynaptic receptors then the α- or β-adrenoceptor blocking drugs would be expected to antagonize this effect. Phentolamine had no significant effect on the convulsions while (±)-propranolol significantly reduced the effects of catechol. Since (±)-propranolol is also a potent membrane stabilizing agent (Seeman, 1972) its effect was compared with (+)-propranolol which has weaker β-blocking activity but similar membrane stabilizing properties. (+)-Propranolol also reduced the convulsive activity, indicating that the results with (±)-propranolol can be explained by local anaesthetic effects. A peripheral site of action was eliminated by the absence of effect of sotalol, a β-adrenoceptor blocking drug which does not cross the blood-brain barrier (Fitzgerald, 1969).

The observations with reserpine, the MAO inhibitors and L-DOPA suggested a non-involvement of dopamine in the central actions of catechol. Apomorphine, a dopamine agonist (Ernst, 1967) and amphetamine which has been shown to release endogenous catecholamines (Thornberg & Moore, 1973), were also found to be ineffective, adding further support to the non-involvement of dopamine.

5-Hydroxytryptamine

Results with drugs causing depletion or elevation of central 5-HT levels were found to be inconsistent. PCPA produces 85% depletion of mouse brain 5-HT with little change in catecholamine levels (Rogers, 1971) and was found to increase significantly the catechol CD_{50}, L-Tryptophan a specific elevator of 5-HT levels (Chase & Murphy, 1973), potentiated the convulsions. Thus, at first sight, a decrease in central 5-HT levels protects against and an increase exacerbates the convulsive activity of catechol.

However, a combination of PCPA to deplete and 5-HTP to restore 5-HT levels (Chase & Murphy, 1973) caused a similar increase in CD_{50} to that obtained with PCPA alone, and methysergide, a 5-HT receptor blocking drug (Douglas, 1970), had no effect on the activity ratio. Further evidence against an involvement of 5-HT in the actions of catechol is given by the lack of effect of reserpine and the MAO inhibitors on the CD_{50} for the convulsions. 5-HT has been implicated in the convulsant action of metrazol, where depletion of the amine with PCPA was found to increase the metrazol seizure susceptibility (cf catechol), and more recently increased brain 5-HT levels have been shown to be associated with infantile myoclonus in guinea-pigs, though this effect could be blocked with methysergide (Klawans, Goetz & Weiner, 1973).

Thus, although the present results do not exclude a tryptaminergic role in the production of catechol convulsions the evidence is equally as good for a non-involvement of 5-HT.

γ-Aminobutyric acid

In an effort to increase cerebral GABA concentrations, animals were pretreated with GABA itself or the GABA-transaminase inhibitor, amino-oxyacetic acid. Neither treatment had any effect on the activity ratio. Thus although GABA is considered likely to be the 'most extensively used inhibitory transmitter in vertebrates' (Krnjević, 1974) it is totally ineffective in modulating the central neuronal activity in response to catechol. Further, the benzodiazepines, clonazepam and librium, which are now thought to act as effective GABA or glycine agonists (Costa, Guidotti, Mao & Suria, 1975) have in preliminary experiments been shown to be ineffective in changing the CD_{50} for catechol convulsions (Angel & Dewhurst unpublished observations).

Acetylcholine

Preliminary experiments had indicated that catechol could act via a cholinergic mechanism since atropine and physostigmine shifted the catechol dose-response curve to the right and left respectively (Angel, 1969). This is supported by the present results in that both muscarinic and nicotinic blocking agents significantly reduce, and anticholinesterases significantly potentiate the convulsions. Interestingly, catechol also causes a depletion of total cerebral acetylcholine from 2.1 μg/g to 1.25 μg/g at the peak of its effect (Angel, unpublished observations), although whether this is cause or effect is not known. Oxotremorine had no significant effect, suggesting that catechol does not stimulate muscarinic receptors directly for which oxotremorine would presumably compete (Koelle, 1970), and that its action is not dependent on elevated cerebral acetylcholine levels, since oxotremorine has been shown to produce a similar increase in brain acetylcholine levels to that caused by physostigmine (Slater & Rogers, 1968) whilst showing no anticholinesterase activity (Holmstedt, Lundgren, Schuberth & Sundwall, 1965).

Whilst the nicotinic blocking agents produced their effects at reasonably low doses, atropine required a relatively high dose (10 mg/kg) to decrease significantly the activity ratio, though the effects of atropine on the convulsions were found to be dose-dependent from 1 to 50 mg/kg (Angel & Dewhurst, unpublished observations). The necessity of a high dose of atropine for the production of central effects appears not to be unusual but a possible 5-HT receptor blockade at higher doses cannot be excluded (Renson, 1971), especially so since the hypermotility and effects on sleep induced by PCPA in unanaesthetized mice is exacerbated by combined PCPA and atropine. treatment (Angel & Dewhurst, unpublished observations). However, it is difficult to reconcile the increased motor excitability in unanaesthetized animals associated with decreased 5-HT

levels with a decrease in excitability in the anaesthetized animal.

Although catechol itself has been shown to possess no anticholinesterase activity (Mogey & Young, 1949) and does not sensitize skeletal muscle acetylcholine receptors to acetylcholine (Blaber & Gallagher, 1971) it does increase the amount of acetylcholine released per nerve impulse at the neuromuscular junction (Otsuka & Nonomura, 1963; Blaber & Gallagher, 1971; Gallagher & Blaber, 1973) and it is possible that a similar presynaptic effect on acetylcholine release is responsible for its central action. This would fit in well with the results for the anticholinesterases and the cholinoceptor blockers and may indicate an action at both muscarinic and nicotinic synapses.

The possibility that the anticholinesterases were acting at the neuromuscular junction was excluded by the observation that in the evoked jerks to somatic stimuli of animals constantly infused with catechol, no signs of iterative muscle discharge were present in the spinal reflex component of the jerk and the increase in muscular contraction was due to an increased amplitude of efferent nerve action potential alone.

In the anaesthetized rat, two surgical procedures, spinal section and dorsal root section reduce the convulsive activity of catechol by 80–85% while a combination of both procedures gives almost total abolition (95%) of its action (Angel & Lemon, 1973). Decerebration, on the other hand, increases its effect some 7 times. Thus, for the spontaneous convulsions, supra-collicular structures inhibit and medullo-ponto-midbrain structures (most probably the brain stem reticular core) appear to be involved in the genesis of the centrifugal activity resulting in spinal motoneuronal activation, but only if their excitability is raised by an increased afferent discharge. Although catechol gives a marked increase in spindle discharge from innervated but not de-efferented muscles, pointing to its central action (Angel, Clarke & Taylor, 1976), the depressant effect of atropine and the excitant effect of physostigmine cannot be of peripheral origin since by themselves they have no effect on muscle spindle discharge. A central site of action for catechol is supported by the lack of effect of atropine methyl nitrate, which does not cross the blood-brain barrier, and the greater potentiation effect of physostigmine compared to neostigmine. Another effect of catechol is to give a marked 'arousal reaction' of electrocortical activity (Angel, 1969; Angel & Lemon, 1974) and it has also been shown to have a marked effect on the discharge of reticular neurones both in the brain stem (Yoshii, Matsumoto & Ogura, 1960) and thalamic reticular nucleus (Angel, 1969). Many cells in the latter structure in the cat are acetylcholine-sensitive (Ben-Ari, Dingledine, Kanazawa & Kelly, 1976) as are cells in the brain stem (see e.g. Bradley & Dray, 1972, 1973) of the rat, and the reticular nuclei contributing to the reticulo-spinal outflow also contain anticholinesterase (Shute & Lewis, 1963; 1965; Holmes & Wolstencroft, 1964).

In conclusion, it would seem that catechol exerts its convulsive effects by facilitating transmission at one or more cholinergic synapses in the central nervous system, possibly by increasing the amount of acetylcholine released per nerve impulse. Its proposed site of action, and the presence of acetylcholine-sensitive neurones at these sites are also in good agreement.

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