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THE EFFECT OF THE CONVULSANT AGENT, CATECHOL, ON NEUROTRANSMITTER UPTAKE AND RELEASE IN RAT BRAIN SLICES

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1 The effect of catechol on uptake and K^+-stimulated release of γ-aminobutyric acid (GABA), D-aspartate, noradrenaline and acetylcholine has been studied in slices of cerebral cortex and thalamus.

2 Low concentrations of catechol did not influence the uptake of any of the neurotransmitters in either brain area.

3 Noradrenaline release was unaffected by catechol.

4 Acetylcholine release from both cortical and thalamic slices was inhibited by high concentrations of catechol. This phenomenon is unlikely to be related to catechol-induced convulsions.

5 Catechol (100 μM) inhibited GABA release from cortical slices by 28%. However, at a concentration of 10 μM catechol enhanced the release of D-aspartate from thalamic slices by over 100%.

6 Potentiated release of excitatory amino acid transmitters may contribute to the enhanced excitability of thalamic cells which occurs during sensory myoclonus induced by low doses of catechol.

Introduction

Low doses of catechol induce in rodents a convulsive state in which non-specific sensory stimulation gives rise to brief myoclonic jerks (Angel & Dawson, 1964); at higher doses this pattern gives way to one of spontaneous convulsions (Angel & Lemon, 1973). Whilst the latter appears to involve an action on the brain stem reticulo-spinal system (Angel & Lemon, 1973) there is evidence that the sensory myoclonic state caused by low doses of catechol may be due to enhanced transmission in the dorsal column sensory pathway at the level of the ventrobasal thalamus. This in turn may be caused by an increased excitatory inflow and a decreased inhibitory inflow to this area from cells outside it, particularly from those in the nucleus reticularis thalami (Angel, 1969).

Pharmacological investigations suggest that both evoked and spontaneous convulsions induced by catechol involve facilitated transmission at some unidentified central cholinergic synapse(s). Catecholamines, 5-hydroxytryptamine (5-HT) and possibly γ-aminobutyric acid (GABA) systems do not appear to be involved (Angel, Clarke & Dewhurst, 1977; Angel & Dewhurst, 1978). Furthermore, at the neuromuscular junction catechol increases the amount of acetylcholine released per nerve impulse (Otsuka & Nonomura, 1963; Blaber & Gallagher, 1971; Gallagher & Blaber, 1973), suggesting that a similar presynaptic action may be responsible for its central effects. This possibility has been investigated in the present experiments; in addition possible presynaptic effects of catechol on other neurotransmitter systems within the thalamus and cerebral cortex have been studied. In regard to the latter, particular attention has been focussed on excitatory amino acid systems since recent neurochemical evidence suggests that some corticothalamic terminals may use these substances as their transmitter (Lund-Karlsen & Fonnum, 1978; Kendall, Minchin & Angel, unpublished observation.)

A preliminary account of some of this work has been published (Minchin, 1981a).

Methods

Release experiments

Adult male albino rats (Sheffield strain) were decapitated and their brains removed on to an ice-cold surface. Frontal slices 2 mm thick were cut on a

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McIlwain tissue chopper and the thalamus was dissected from them; cerebral cortex was dissected from whole brain. The tissue from each area was then chopped into small ($0.1 \times 0.1 \times 2.0$ mm) slices and following a 10 min preincubation at 37°C either 50 mg (cortex) or 25 mg (thalamus) portions were incubated with (a) D-[2,3-^{3}H]-aspartic acid (Radiochemical Centre, Amersham, sp. act. 18 Ci/mmol, final concentration 22 nM) plus [U-^{14}C]-GABA (Radiochemical Centre, Amersham, sp. act. 224 mCi/mmol, final concentration 0.45 μM) for 15 min, (b) DL-[7-^{3}H]-noradrenaline (Radiochemical Centre, Amersham, sp. act. 10 Ci/mmol, with unlabelled noradrenaline to give final concentration of 0.1 μM) for 15 min or (c) [^{14}C-methyl]-choline (Radiochemical Centre, Amersham, sp. act. 59 mCi/mmol, final concentration 3.4 μM) for 30 min. Following incubation the slices were collected by filtration on to Whatman GF/A filters mounted in Sartorius membrane filter holders and superfused with warm, oxygenated Krebs-phosphate solution (composition, mM: NaCl 118, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 1.2, D-glucose 5.6 and sodium phosphate buffer, pH 7.4, 15) at a rate of 0.5 ml/min. Metabolism of GABA was prevented by the addition to the medium of amino oxyacetic acid 10 μM; D-aspartate is not metabolised by brain tissue although it is taken up by the same high affinity system as L-aspartate and probably L-glutamate. Noradrenaline metabolism was minimized by superfusion with medium containing ascorbic acid 0.1 mg/ml and nialamide 10 μM, whilst hydrolysis of [^{14}C]-acetylcholine formed from [^{14}C]-choline was prevented by the presence in the superfusion medium of 100 μM physostigmine sulphate. After a 20 min period, during which extracellular radioactivity was washed out from the tissue, fractions of superfusate were collected every 3 min. The radioactivity in each fraction and that remaining in the tissue at the end of the experiment was estimated by liquid scintillation spectrometry and the former expressed as an approximate first order rate constant (Minchin, 1981b). Tissue was depolarized by exposure to 40 mM K^+ (as KCl replacing NaCl) for 9 min. In each experiment 3 samples were depolarized in the absence of drug (controls) and a further 3 samples, run in parallel, were exposed to added drugs for 6 min before and during depolarization. The increase in release caused by K^+ in the presence of added drugs was expressed as a percentage of the mean increase found in controls and the normalized data were analysed by the Mann-Whitney U test.

Uptake experiments

Slices (10 mg) were preincubated in 10 ml Krebs-phosphate solution at 37°C for 10 min after which (a) [^{14}C]-GABA plus D-[^{3}H]-aspartate (final concentrations 11 nM and 0.2 nM, respectively, (b) [^{3}H]-noradrenaline (final concentration 0.1 μM) or (c) [^{14}C]-choline (final concentration 0.1 μM) were added and the incubation continued for (a) 10 min, (b) 6 min or (c) 5 min. The slices were then collected by filtration on to Whatman No. 1 filters, rinsed with 5 ml Krebs-phosphate, extracted with 1.4 ml water and counted by liquid scintillation spectrometry. Catechol, when present, was added at the start of the preincubation period. Blanks were run in which preincubation and incubation took place at 0°C. Metabolic inhibitors were present in the incubation medium at the same concentrations as in the release experiments.

[^{14}C]-choline metabolism

Slices were incubated with [^{14}C]-choline and superfused as described above, following which pooled fractions taken before, during and after exposure to 40 mM K^+ were subjected to liquid cation exchange and high voltage electrophoresis. At the end of the superfusion the tissue was homogenized in 8% formic acid and similarly treated. Choline esters were extracted from 4 ml samples, to which 20 μg each of choline and acetylcholine had been added as carriers, by treatment with 1 ml tetraphenylboron in 3-heptanone (10 mg/ml, except the 40 mM K^+ fractions which were treated with 5 mg/ml to avoid precipitation of potassium tetraphenylboron). The organic phase was separated from the aqueous and shaken with 250 μl 400 mM HCl; this procedure back-extracts choline esters into the aqueous phase. Samples of the acid aqueous phase were then spotted on to Whatman 3MM paper and subjected to electrophoresis in 8% formic acid (2200 V for 45 min). The paper was dried and choline esters were visualized with iodine vapour. The chromatographs were cut into strips which were extracted with 1.3 ml water and counted in a liquid scintillation spectrometer. Known quantities of [^{14}C]-choline were taken through this procedure to establish the percentage recovery; the percentage recovery of acetylcholine was taken as twice that of choline (Fonnum, 1969).

Results

The effect of catechol on transmitter uptake

At low concentrations catechol had no effect on transmitter uptake (Table 1). However, catechol at 500 μM weakly inhibited uptake of all the transmitters tested except that of D-aspartate into cortical slices (Table 1).

Table 1 The effect of catechol on transmitter uptake in rat brain slices

Transmitter	Catechol (μM)	Uptake (% of control) Cortex	Thalamus
D-Aspartate	10	—	99 ± 5
	100	106 ± 9	88 ± 4
	500	90 ± 2	66 ± 4 (3)†††
GABA	10	—	100 ± 3
	100	96 ± 5	98 ± 2
	500	87 ± 1††	82 ± 6†
Choline	500	73 ± 8†	77 ± 6††
Noradrenaline	500	64 ± 6††	—

Figures represent the mean ± s.e.mean of 4 experiments except where indicated.
† $P<0.05$, †† $P<0.02$, ††† $P<0.01$ } 2-tailed t test

Ca^{2+} and K^+-stimulated transmitter release

The potassium-stimulated release of both GABA and D-aspartate from cerebral cortex slices has previously been shown to be mostly calcium-dependent (Minchin, 1981b). Similarly, when Ca^{2+} was removed from the medium superfusing thalamic slices and the Mg^{2+} concentration raised to 10 mM the K^+-stimulated release of GABA was reduced to 44 ± 6% (s.e.mean, $n=5$) of control and that of D-aspartate to 43 ± 11% (s.e.mean, $n=5$) of control. In the case of noradrenaline release from cortex slices the same procedure depressed the K^+-stimulated release to 14% of control (mean of 2 experiments). Figure 1 shows that all of the K^+-stimulated, and possibly some of the spontaneous efflux of acetylcholine from both cortex and thalamic slices was abolished by removing Ca^{2+} from the superfusing medium and raising the Mg^{2+} concentration to 10 mM.

Catechol and amino acid release

At the highest concentration tested (500 μM) catechol caused a slight (10–20%) enhancement of spontaneous GABA release from both cortical and thalamic slices. In thalamic slices catechol had no significant effect upon the K^+-stimulated release of GABA at any of the concentrations tested. By contrast however, low concentrations of catechol enhanced K^+-stimulated D-aspartate release by over 100%; very low and high concentrations had no effect (Figure 2). The 3-hydroxy derivative of catechol, pyrogallol, had no effect upon the release of either D-aspartate or GABA at the concentration at which catechol had maximum effect on D-aspartate release (Figure 2).

In cerebral cortex slices, catechol had a different effect on amino acid release. At intermediate concentrations it inhibited GABA release by 28%; high and low concentrations were without effect

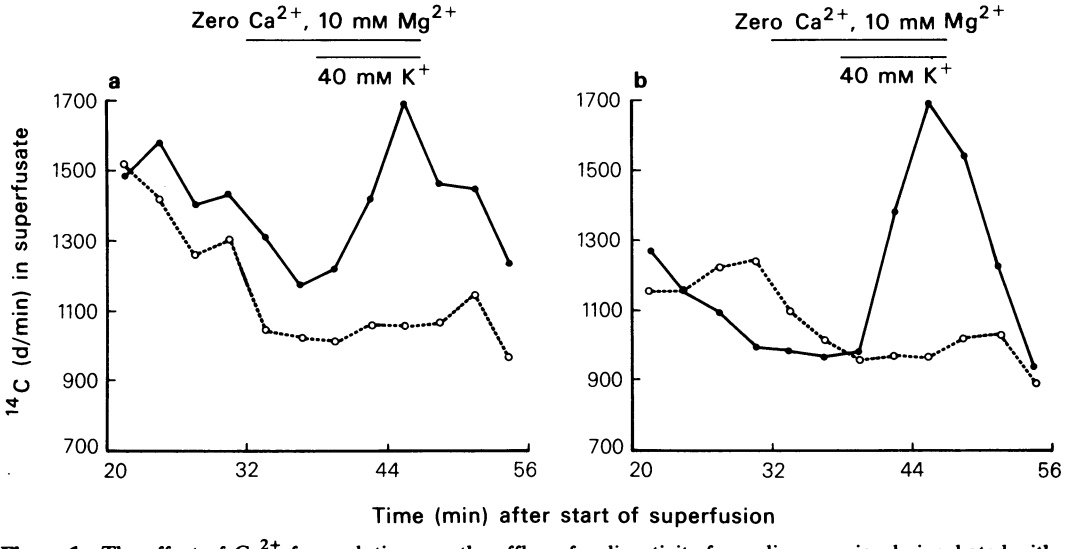

Figure 1 The effect of Ca^{2+}-free solutions on the efflux of radioactivity from slices previously incubated with [^{14}C]-choline. Slices of cortical (a) or thalamic (b) tissue were incubated with [^{14}C]-choline and superfused as described in Methods. Control slices were depolarized with 40 mM K^+ for the period shown by the short horizontal line whilst experimental slices were superfused with Ca^{2+}-free medium, as shown by the longer horizontal line, both before and during depolarization. (●) Control; (○) Ca^{2+}-free. Each point is the mean of 3 experiments.

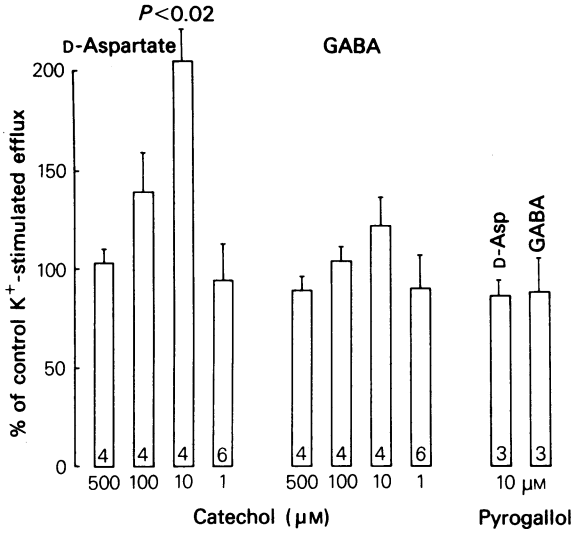

Figure 2 The effect of catechol and pyrogallol on K^+-stimulated release of [^{3}H]-D-aspartate and [^{14}C]-GABA from thalamic slices. Tissue was incubated and superfused as described in Methods. Spontaneous efflux was approximately 0.1% of tissue stores per min for aspartate and 0.05% of tissue stores per min for GABA. Control slices were depolarized in the absence of drug, experimental slices were superfused with drug from 6 min before and during depolarization. K^+-stimulated release was calculated as the percentage increase in efflux rate constant seen during exposure to 40 mM K^+. In pooled controls this increase was 99±9% (mean±s.e.mean, $n=19$) for D-aspartate and 664±50% (mean±s.e.mean, $n=19$) for GABA. The K^+-stimulated release seen in the presence of drug was expressed as a percentage of controls run at the same time. Each column represents the mean of the number of experiments indicated within the column; vertical lines show s.e.mean. Statistical analysis was by Mann-Whitney U test.

(Figure 3). The release of D-aspartate was slightly, but not significantly, depressed at intermediate concentrations and pyrogallol again failed to alter the release of either amino acid at the concentration at which catechol inhibited GABA release (Figure 3).

Catechol and noradrenaline release

Catechol had no significant effect upon K^+-stimulated noradrenaline release at any of the concentrations tested (Figure 4).

Catechol and acetylcholine release

Liquid cation exchange and electrophoresis of the superfusate solutions from cortex slices that had been incubated with low concentrations of [^{14}C]-choline demonstrated that before depolarization, 12% of the radioactivity in the superfusate was due to acetylcholine whilst during exposure to 40 mM K^+ this figure increased to 32%. Of the radioactivity in the tissue, 51% was acetylcholine. Before depolarization of thalamic slices, 7% of the radioactivity in the superfusate was due to acetylcholine whilst depolarization increased this to 27%. Of the tissue radioactivity, 45% was present as acetylcholine.

These figures were used to correct the radioactivity appearing in the superfusate solutions to give the amount present as acetylcholine. Figure 5 shows that high concentrations of catechol inhibited the K^+-stimulated release of acetylcholine from both thalamic and cortical slices, although owing to the scatter in control values only the former change reached significance. Lower concentrations of catechol were without effect in either tissue and high concentrations of pyrogallol were ineffective in the cortex.

Discussion

The results of the present study indicate that changes in the uptake of aspartate, GABA, choline or noradrenaline do not appear to be associated with the sensory myoclonic jerks seen following low doses of catechol. The weak inhibition seen at high catechol concentration may be related to the spontaneous convulsions that occur after higher doses, but the lack of specificity with regard to both transmitter and

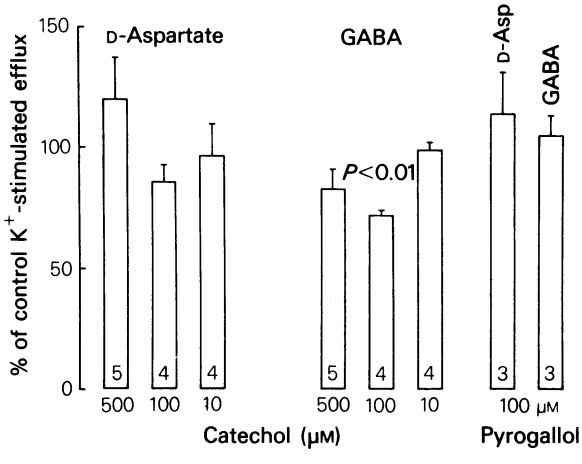

Figure 3 The effect of catechol and pyrogallol on K^+-stimulated release of [^{3}H]-D-aspartate and [^{14}C]-GABA from cortical slices. Experimental protocol is described in the legend Figure 2. Spontaneous efflux was approximately 0.1% of tissue stores per min for both aspartate and GABA. In pooled controls, 40 mM K^+ caused a 164±15% (mean±s.e.mean, $n=19$) increase in the rate constant for D-aspartate and a 734±72% (mean±s.e.mean, $n=19$) increase for GABA.

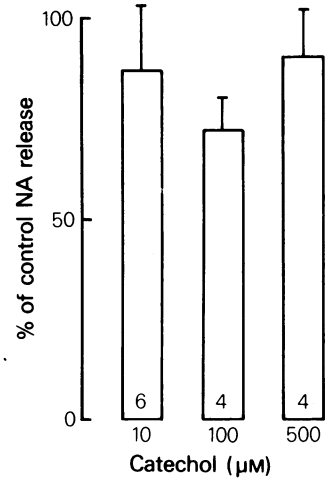

Figure 4 The effect of catechol on [^{3}H]-noradrenaline (NA) release from cortical slices. The experimental protocol was identical to that described in the legend to Figure 2. Spontaneous efflux was approximately 0.9% of tissue stores per min. In pooled controls, 40 mM K^+ caused a 119 ± 13% (mean ± s.e.mean, $n = 12$) increase in the efflux rate constant for noradrenaline.

brain region make this unlikely. Alterations in uptake did not influence release.

Since there is very little noradrenaline present in the thalamus (Fuxe, Hökfelt & Ungerstedt, 1969) its transport was not studied in this area. However, noradrenaline release from the cortex was Ca^+-dependent and therefore, presumably, a neuronal phenomenon. Furthermore it was unaffected by catechol and is therefore not a causative factor in the convulsions produced by this substance. This is consistent with the results of experiments which demonstrated that drugs altering catecholamine function were without effect on catechol-induced convulsions (Angel *et al.*, 1977; Angel & Dewhurst, 1978).

Neurophysiological evidence indicates that cortical excitability is unchanged after low doses of catechol, apart from a transient increase that is probably due to hyperpnoea (Angel, 1969), and in the present study cortical release of D-aspartate, GABA and acetylcholine was unaffected by low concentrations of catechol. D-Aspartate transport is also a measure of the transport of L-aspartate and/or L-glutamate and since these amino acids, in addition to acetylcholine and GABA, probably act as transmitters within the cerebral cortex (Lewis & Shute, 1967; Curtis & Johnston, 1974) the physiological and neurochemical lines of evidence converge.

GABA is an inhibitory transmitter and so the reduction, though small, seen in cortical GABA release at high catechol concentration might be expected to result in some enhancement of cortical excitability. However, acetylcholine and aspartate are predominantly excitatory within the cerebral cortex (Crawford & Curtis, 1966; Curtis & Johnston, 1974) and so the tendency towards inhibition of their release at high catechol concentrations might be expected to counteract, at least in part, the influence of a reduction in GABA release. Pharmacological experiments have demonstrated that amino oxyacetic acid-induced elevation of brain GABA levels had no effect on catechol-induced, spontaneous convulsive activity (Angel *et al.*, 1977). However, evoked GABA release is not altered when GABA levels are raised by amino oxyacetic acid (Iversen, Mitchell & Srinivasan, 1971), so under these circumstances catechol might still be able to depress GABA release. Nevertheless, benzodiazepines also fail to modify catechol convulsions (Angel *et al.*, 1977) and since benzodiazepines potentiate GABA action (Simmonds, 1980), on balance the evidence suggests that GABA is not involved in the genesis of these convulsions.

In thalamic slices catechol at high concentrations had no effect on the release of GABA or aspartate, although acetylcholine release was depressed. The latter finding is unlikely to be related to the convulsive activity induced by catechol for two reasons.

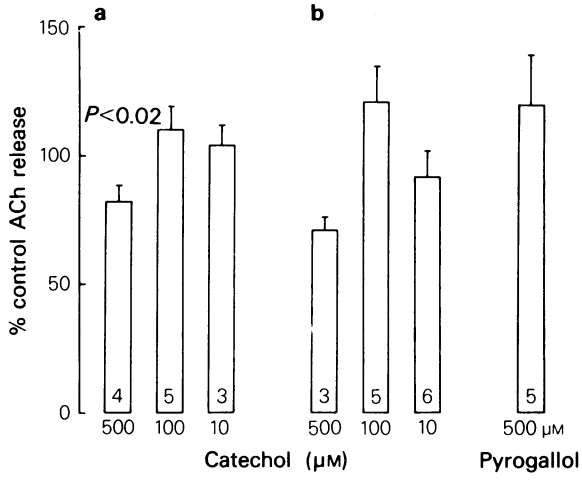

Figure 5 The effect of catechol and pyrogallol on K^+-stimulated [^{14}C]-acetylcholine (ACh) release from slices of thalamus (a) and cortex (b). The experimental protocol is described in the legend to Figure 2; the amount of radioactivity present in the superfusing solution as acetylcholine was estimated as described in Methods. Spontaneous efflux was approximately 0.4% of tissue stores of acetylcholine per min from cortex and 0.2% of tissue stores per min from thalamus. In pooled controls, depolarization caused a 395 ± 12% (mean ± s.e.mean, $n = 10$) increase in acetylcholine efflux in thalamic slices and a 366 ± 30% (mean ± s.e.mean, $n = 16$) increase in cortical slices.

Firstly the concentration of catechol within the brain following a dose of 60 mg/kg (i.p.) that induced spontaneous convulsions was found to be 34 μg/g wet wt (Rogers, Angel & Butterfield, 1968). If it is assumed that catechol distributes throughout the total brain water this corresponds to a concentration of approximately 380 μM, which is somewhat lower than that at which acetylcholine release is depressed. Secondly, spontaneous catechol convulsions are inhibited by cholinoceptor blocking agents and potentiated by anticholinesterases (Angel *et al.*, 1977) which implies that cholinergic function is enhanced, not depressed, by catechol under these circumstances.

Low concentrations of catechol had no significant effect upon the release of acetylcholine or GABA from thalamic slices but potentiated aspartate release by over 100%. Since aspartate and/or glutamate may be the transmitters of some cortico-thalamic terminals (Lund-Karlsen & Fonnum, 1978; Kendall, Minchin & Angel, unpublished observation) it is possible that this large potentiation of excitatory amino acid function may contribute to the enhanced excitability of thalamic cells which occurs following low doses of catechol and which has been suggested to be due, at least in part, to increased excitatory inflow to thalamic relay cells (Angel, 1969).

One possible explanation for the catechol-induced changes in transmitter release seen in this study is not that they are related to the convulsions induced by catechol but rather that they result from the depression of brain ATP levels that accompanies catechol treatment. However, this is not so since pyrogallol, which produces a similar fall in cerebral ATP to that of catechol yet has no convulsive activity (Angel, Lemon, Rogers & Banks, 1969), had no effect upon transmitter release in the present experiments.

Previous experiments have shown that at the neuromuscular junction, micromolar concentrations of catechol increased the release of acetylcholine (Otsuka & Nonomura, 1963; Gallagher & Blaber, 1973), but that high concentrations (100 μM) inhibited release (Gallagher & Blaber, 1973). This study has extended these findings to central acetylcholine release only in the case of high concentrations of catechol and despite the pharmacological evidence indicating a catechol-induced enhancement of central acetylcholine function (Angel *et al.*, 1977; Angel & Dewhurst, 1978) no potentiation of release at low catechol concentration was detected in the present study. Although catechol does not sensitize the postsynaptic receptor to acetylcholine at the neuromuscular junction (Otsuka & Nonomura, 1963) it is possible that such an interaction occurs in the CNS.

In conclusion, the results of the present study demonstrate that in addition to the enhancement of acetylcholine function postulated on pharmacological grounds, there is a potentiation of thalamic presynaptic excitatory amino acid release that may contribute to the increased excitability of cells within the thalamus following low doses of catechol.

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References

ANGEL, A. (1969). An analysis of the effect of 1,2-dihydroxybenzene on transmission through the dorsal column sensory pathway. *Electroenceph. clin. Neurophysiol.*, **27**, 392–403.

ANGEL, A. & DAWSON, G.D. (1964). The effect of 1,2-dihydroxybenzene on transmission in the sensory pathway of the rat. *Electroenceph. clin. Neurophysiol.*, **16**, 312.

ANGEL, A. & LEMON, R.N. (1973). The convulsive action of 1,2-dihydroxybenzene in the anaesthetised rat. *Electroenceph. clin. Neurophysiol.*, **34**, 369–378.

ANGEL, A. & DEWHURST, D.G. (1978). A pharmacological investigation of the electrically evoked convulsive activity induced by administration of catechol in the anaesthetized rat. *Br. J. Pharmac.*, **64**, 539–544.

ANGEL, A., CLARKE, K.A. & DEWHURST, D.G. (1977). A pharmacological study of the spontaneous convulsive activity induced by 1,2-dihydroxybenzene (catechol) in the anaesthetized mouse. *Br. J. Pharmac.*, **61**, 433–439.

ANGEL, A., LEMON, R.N., ROGERS, K.J. & BANKS, P. (1969). The effect of polyhydroxyphenols on brain ATP in the mouse. *Exp. Brain Res.*, **7**, 250–257.

BLABER, L.C. & GALLAGHER, J.P. (1971). The facilitatory effects of catechol and phenol at the neuromuscular junction of the cat. *Neuropharmac.*, **10**, 153–159.

CRAWFORD, J.M. & CURTIS, D.R. (1966). Pharmacological studies on feline Betz cells. *J. Physiol.*, **186**, 121–138.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69**, 97–188.

FONNUM, F. (1969). Isolation of choline esters from aqueous solutions by extraction with sodium tetraphenylboron in organic solvents. *Biochem. J.*, **113**, 291–298.

FUXE, K., HÖKFELT, T. & UNGERSTEDT, U. (1969). Distribution of monamines in the mammalian central nervous system by histochemical studies. In: *Metalolism of Amines in the Brain.* ed. Hooper, G., pp. 10–22. London: Macmillan.

GALLAGHER, J.P. & BLABER, L.C. (1973). Catechol, a facilitatory drug that demonstrates only a prejunctional site of action. *J. Pharm. exp. Ther.*, **184**, 129–135.

IVERSEN, L.L., MITCHELL, J.F. & SRINIVASAN, V. (1971). The release of γ-aminobutyric acid during inhibition in the cat visual cortex. *J. Physiol.*, **212**, 519–534.

LEWIS, P.R. & SHUTE, C.C.D. (1967). The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system, and the subfornical organ and supra-optic crest. *Brain*, *90*, 521–540.

LUND-KARLSEN, R. & FONNUM, F. (1978). Evidence for glutamate as a neurotransmitter in the corticofugal fibres to the dorsal lateral geniculate body and the superior colliculus in rats. *Brain Res.*, **151**, 457–467.

MINCHIN, M.C.W. (1981a). The effect of catechol on amino acid transmitter release from thalamic slices. *Br. J. Pharmac.*, **73**, 234P.

MINCHIN, M.C.W. (1981b). The effect of anaesthetics on the uptake and release of γ-aminobutyrate and D-aspartate in rat brain slices. *Br. J. Pharmac.*, **73**, 681–689.

OTSUKA, M. & NONOMURA, Y. (1963). The action of phenolic substances on motor nerve endings. *J. Pharm. exp. Ther.*, **140**, 41–45.

ROGERS, K.J., ANGEL, A. & BUTTERFIELD, L. (1968). The penetration of catechol and pyrogallol into mouse brain and the effect on cerebral monoamine levels. *J. Pharm. Pharmac.*, **20**, 727–729.

SIMMONDS, M.A. (1980). A site for the potentiation of GABA-mediated responses by benzodiazepines. *Nature*, **284**, 558–560.

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