THE SPASMOLYTIC ACTIONS OF PYROGALLOL AND CATECHOL ON THE ISOLATED GUINEA-PIG ILEUM

BY E. S. JOHNSON

From the Department of Pharmacology, King's College, Strand, London, W.C.2

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Pyrogallol and catechol, potent inhibitors of catechol-*o*-methyl transferase, have general non-specific spasmolytic actions on the isolated guinea-pig ileum. Spasmogens acting by the intramural nervous mechanism are inhibited by lower concentrations of pyrogallol and catechol than are spasmogens acting directly on the smooth muscle. Possible explanations and implications are discussed.

CATECHOL-*o*-METHYLTRANSFERASE is a principal pathway for the metabolism and inactivation of adrenaline and noradrenaline (Axelrod and Laroche, 1959; Axelrod, 1960) and pyrogallol and catechol are potent inhibitors of this enzyme (Axelrod and Laroche, 1959; Axelrod, 1960; Bacq, 1959; Wylie, Archer and Arnold, 1960). They have been shown to potentiate the actions of sympathomimetic amines (Axelrod, 1960; Wylie and others, 1960; Bacq, 1936; Izquierdo and Izquierdo, 1961) by the inhibition of the *o*-methyltransferase, but Barger and Dale (1910–11) concluded that catechol had no sympathomimetic effect but merely a direct tonic action on smooth muscle fibres.

Recently, completely different actions of pyrogallol and catechol have been described. Sjöstrand (1960; 1961) investigated the actions of catechol on the isolated terminal ileum of the guinea-pig; he reported that doses between 0·5 and 5·0 mg. (in a 15 ml. bath) usually produced a stimulation thought to be caused by the stimulation of ganglia in the walls of the intestine. Tachyphylaxis observed with consecutive doses was described as a secondary nicotine-like persistent depression of the ganglia. Higher doses of catechol inhibited contractures induced by acetylcholine, histamine, and substance P.

Izquierdo and Izquierdo (1961) found that pyrogallol produced an initial depressor followed by a stimulatory effect on the motility of the dog duodenum. They claimed the depressor action was adrenaline-like and thought that the stimulation was cholinergic.

Jaques and Doepfner (1959) showed that lysis of the spasm induced by different spasmogens on visceral smooth muscle was a general property possessed by phenol and many of its derivatives. Although pyrogallol and catechol were not investigated the 1,3- and 1,4-isomers of catechol were found to have spasmolytic actions.

It seems important to question the specificity of the *o*-methyl transferase activity of pyrogallol and catechol and to enquire into the site of other actions on smooth muscle.

METHODS

Adult female guinea-pigs of 600 to 750 g. were killed by a blow on the head and bled. The ileum was excised and 3 cm. segments from the

middle and terminal regions were removed and mounted with the oral end downwards in a 10 ml. bath containing Krebs's solution aerated with 95 per cent O_2 and 5 per cent CO_2. Longitudinal contractures were recorded isotonically. Pyrogallol or catechol dissolved in Krebs's solution replaced the bathing medium 10 min. before the dose-response curves were repeated.

The concentrations of pyrogallol were 1×10^{-6}, 5×10^{-6}, 1×10^{-5} and 5×10^{-5}, those of catechol were 1×10^{-4}, 5×10^{-4} and 1×10^{-3} but some intermediate concentrations were also used. The solutions were protected from light.

Dose-response curves were induced by the following spasmogens: acetyl-β-methylcholine chloride (methacholine), 5-hydroxytryptamine creatinine sulphate (5-HT), choline phenyl ether bromide (TMI), dimethylphenylpiperazinium iodide (DMPP) and histamine acid phosphate. All the drug concentrations were expressed as μg. base/ml.

RESULTS

Pyrogallol

Pyrogallol, 1 μg. to 5 mg., did not contract the ileum. In doses of 1×10^{-6} pyrogallol had no effect on the dose-response curves of the spasmogens; doses of 5×10^{-6} usually reduced the responses to 5-HT, TMI and DMPP but did not modify the methacholine and histamine curves. 1×10^{-5} pyrogallol usually inhibited both methacholine and histamine at all doses, with the effect of displacing the dose-response curve, while the 5-HT, TMI and DMPP effects were almost abolished; 5×10^{-5} pyrogallol always abolished the 5-HT, TMI and DMPP effects and reduced the methacholine and histamine responses to such an extent that their former maxima were unattainable (Fig. 1).

In some experiments it was found that a concentration of 1×10^{-5} pyrogallol had very little effect whereas 5×10^{-5} had the effect described above, that is, abolition of the 5-HT, TMI and DMPP responses and a marked reduction of those to methacholine and histamine. In these experiments it was found that intermediate concentrations ($2{\cdot}5 \times 10^{-5}$) reduced only the 5-HT, TMI and DMPP responses.

In a single experiment, barium chloride and carbachol behaved in the same way as histamine and methacholine.

The preparation was washed with normal Krebs's solution for 15 to 30 min. after each concentration of pyrogallol and the normal responses were obtained again.

Catechol

When doses of catechol between 0·1 and 5 mg. were added to the bath no stimulation was recorded. An ED70 of histamine was injected in between each dose of catechol to monitor any possible persistent depressant action. But when the catechol Krebs's solution was used as the bathing medium, an initial longitudinal contracture was sometimes seen which returned to the baseline after one minute.

Qualitatively similar results to those with pyrogallol were obtained with catechol, the most important difference being that an approximately ten-fold higher concentration of catechol was required to produce the same effects as pyrogallol. Thus a concentration of 1×10^{-4} catechol hardly affected the dose response curves to any spasmogen. At 5×10^{-4}, it reduced those of 5-HT, TMI and DMPP to the extent that their former maxima were unattainable; it inhibited the histamine effects, displacing the dose-response curve, but left the methacholine curve unchanged. 1×10^{-3} catechol abolished the 5-HT, TMI and DMPP contractures, greatly reduced the histamine maximum and displaced the methacholine dose-response curve leaving its maximum unchanged (Fig. 2).

The normal responses returned after washing with normal Krebs's solution for 30 min.

Pyrogallol and catechol had the same actions on both middle and terminal parts of the guinea-pig ileum.

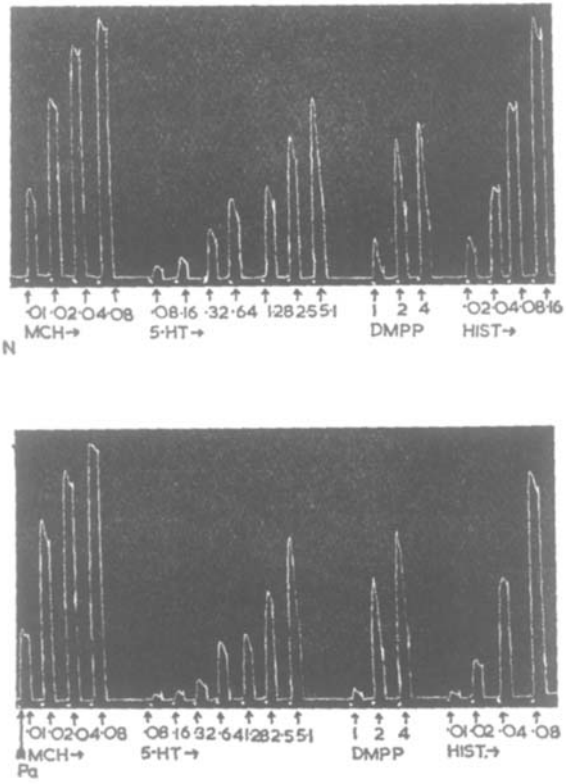

FIG. 1A. The spasmolytic actions of pyrogallol on the guinea-pig terminal ileum.
N = Dose response curves with normal Krebs's solution.
Pa = Dose response curves with Krebs's containing 1×10^{-6} pyrogallol.

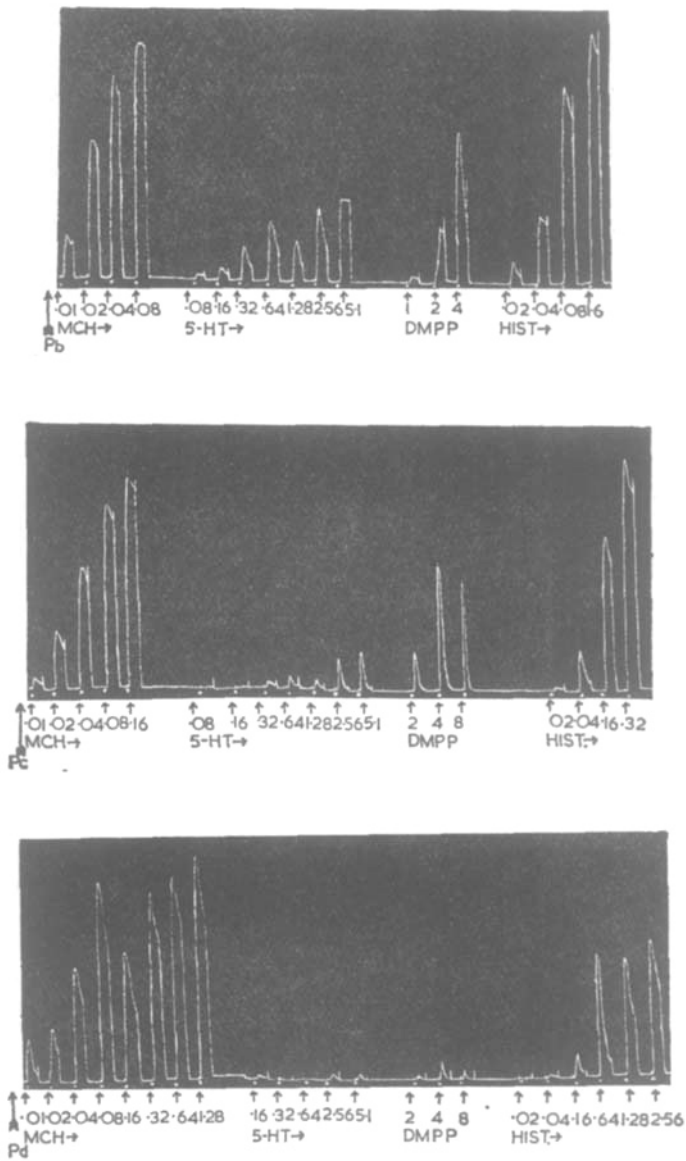

FIG. 1B. The spasmolytic actions of pyrogallol on the guinea-pig terminal ileum.
Pb = Dose response curves with Krebs's containing 5×10^{-6} pyrogallol.
Pc = Dose response curves with Krebs's containing 1×10^{-5} pyrogallol.
Pd = Dose response curves with Krebs's containing 5×10^{-5} pyrogallol.

Doses of spasmogens in μg./ml. bath volume.
Bath temperature 37°.
Contact time of the spasmogens 30 sec., doses repeated every 2 min.

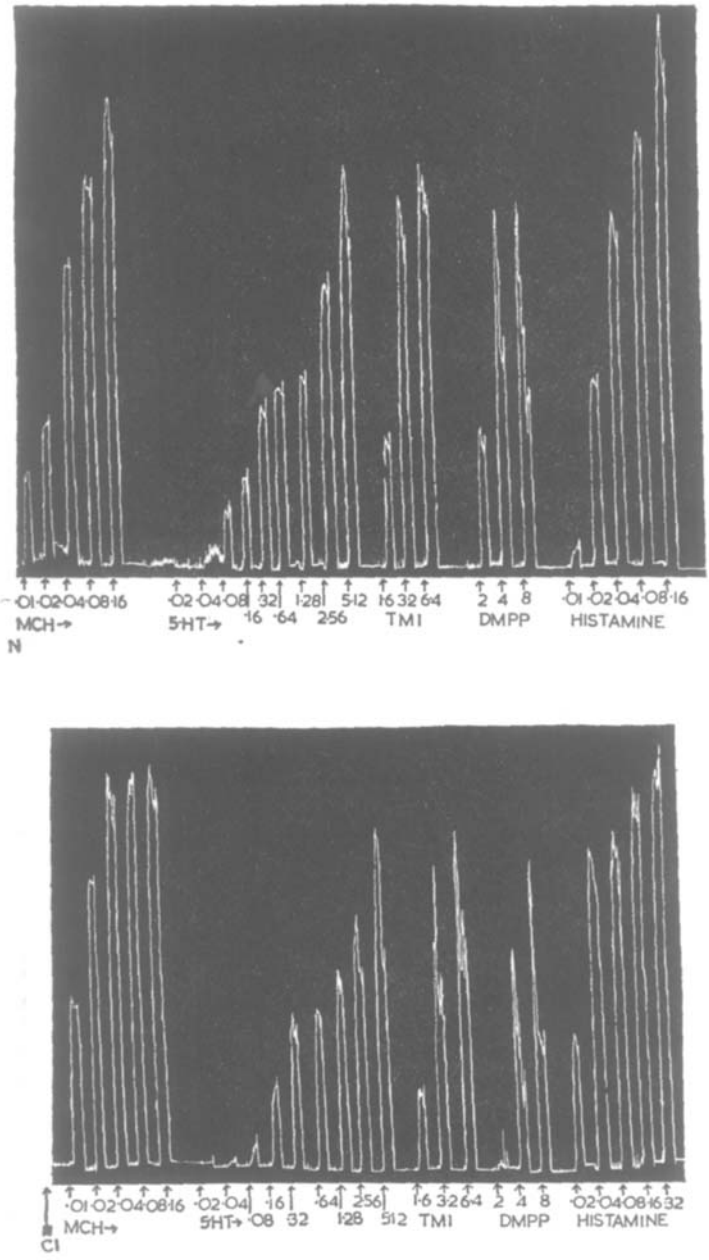

FIG. 2A. The spasmolytic actions of catechol on the guinea-pig terminal ileum.
N = Dose-response curves with normal Krebs's solution.
C1 = Dose-response curves with Krebs's containing 1×10^{-4} catechol.

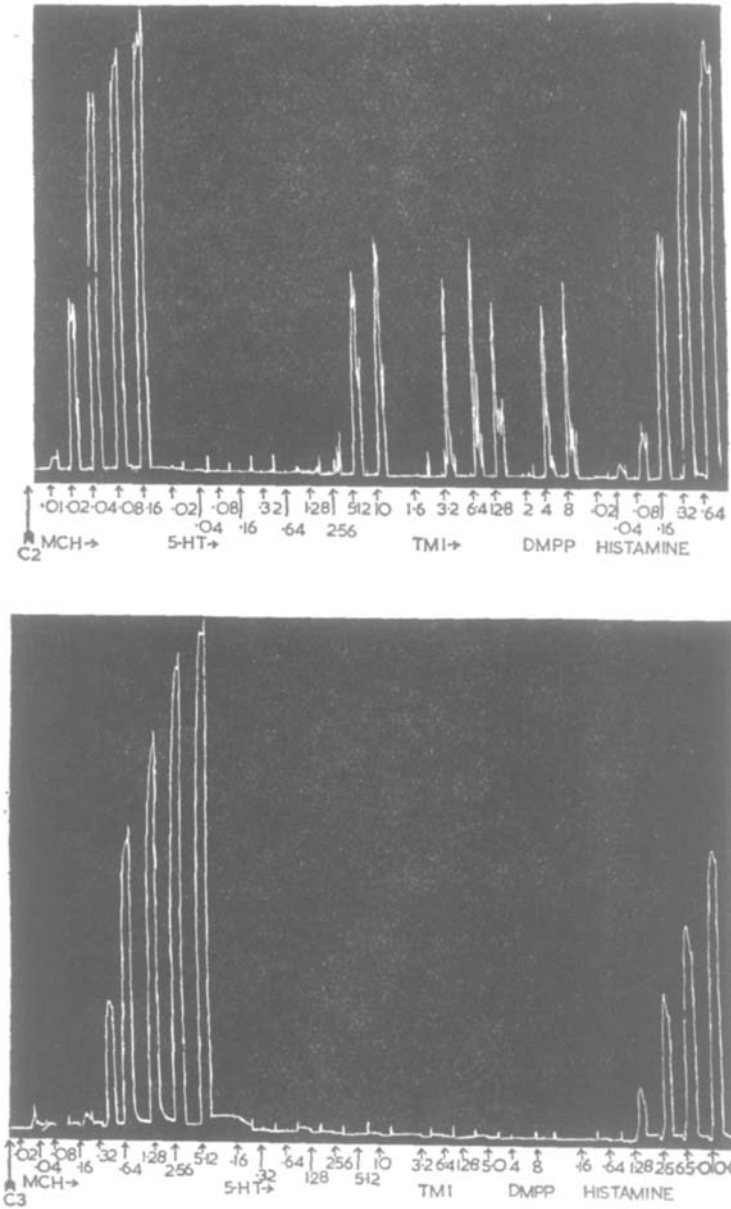

FIG. 2B. The spasmolytic action of catechol on the guinea-pig terminal ileum.
C2 = Dose-response curves with Krebs's containing 5×10^{-4} catechol.
C3 = Dose-response curves with Krebs's containing 1×10^{-3} catechol.

Doses of spasmogens in μg/ml. bath volume.
Bath temperature 37°.
Contact time of the spasmogens 30 sec., doses repeated every 2 min.

Discussion

Pyrogallol and catechol are derivatives of phenol and the discovery that these compounds produce lysis of drug-induced spasm is in agreement with the results of Jaques and Doepfner (1959) who investigated phenol and some of its derivatives. They concluded that phenol-like compounds possessed a general spasmolytic property on smooth muscle similar to that of papaverine.

The present experiments show that contractures induced by 5-HT, TMI and DMPP, which are believed to act by nervous pathways (Rocha e Silva, Valle and Picarelli, 1953; Gaddum and Picarelli, 1957; Hey, 1952; Leach, 1957; Ling, 1959), are inhibited by concentrations of pyrogallol and catechol which have little or no effect on contractures induced by histamine and methacholine. A possible explanation of this action resides in the local anaesthetic property common to phenols.

Because the action of pyrogallol and catechol in modifying the spasmogenic effects of all the spasmogens used, is reversible, they cannot be designated toxic but are more like the non-specific spasmolytic drug papaverine.

In experiments designed to investigate the possible stimulatory actions of catechol, no positive results were obtained and so the discovery of Sjöstrand (1960; 1961) that this compound acts by stimulating nicotinic receptors remains unconfirmed. The occasional stimulation seen with my experiments when normal Krebs's solution was replaced by Krebs's containing catechol may have been caused by the difference in temperature of the latter solution when first introduced into the bath. The inhibitory concentrations of catechol were in the same dose region as the inhibitory concentrations employed by Sjöstrand and his observations in this respect are confirmed.

Izquierdo and Izquierdo (1961) noted an initial depression of motility of the dog duodenum *in situ* with pyrogallol in the same dose ranges as those used in this report. It seems likely that part of this inhibition was caused by the general spasmolytic property of pyrogallol.

These experiments draw attention to the dangers of relying on pyrogallol and catechol as specific catechol-*o*-methyl transferase inhibitors. The general spasmolytic properties of these compounds can not be disregarded in assessing their actions on smooth muscle.

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