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CATECHOL-O-METHYL TRANSFERASE INHIBITION AND POTENTIATION OF EPINEPHRINE RESPONSES BY DESMETHYLPAPAVERINE

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Abstract—Papaverine was demethylated chemically to yield desmethylpapaverine (papaveroline).

In low and intermediate concentrations it moderately potentiated contractions, of isolated aortic strip of rabbit, induced by epinephrine, angiotensin, and potassium (nonspecific potentiation); in higher concentrations it markedly potentiated the epinephrine-induced contractions only (specific potentiation). On the blood pressure of the anaesthetized cat desmethylpapaverine potentiated epinephrine-induced pressor responses and prolonged isoproterenol-induced depressor responses.

In vitro studies of catechol-O-methyl transferase (COMT) indicate that desmethylpapaverine is a potent inhibitor of the enzyme. It is suggested that the specific potentiation produced by desmethylpapaverine may be due to inhibition of COMT.

WHILE investigating the pharmacological properties of the catechol-O-methyl transferase (COMT) inhibitor, 4-methyltropolone,[1] it was noted that in low concentrations it potentiated epinephrine-induced contractions of the guinea pig's vas deferens but in high concentrations antagonized them. This compound was subsequently shown to exert a spasmolytic action against a variety of spasmogens on several test organs. When papaverine was tested on the vas deferens it inhibited epinephrine-induced contractions, but to our surprise it often initially potentiated them when applied in a low concentration. Axelrod[2] had demonstrated that demethylases are capable of demethylating papaverine. It therefore seemed possible that perhaps papaverine was being demethylated to form a catechol-like compound which would then potentiate epinephrine by inhibiting the enzyme largely responsible for its destruction, i.e. COMT. Consequently desmethylpapaverine [6,7-dihydroxy-1-3,4-dihydroxybenzylisoquinoline] or papaveroline was prepared by chemical removal of the four methyl groups to give a compound with two pairs of adjacent hydroxyls. This compound was then tested pharmacologically on isolated tissue, the anaesthetized animal, and enzymatically on COMT.

METHODS

Pharmacological

The aortic helical strip of the rabbit,[3] 3 mm wide and 50 mm long when stretched and weighing approximately 90 mg, was bathed in Krebs-Henseleit solution at 37°

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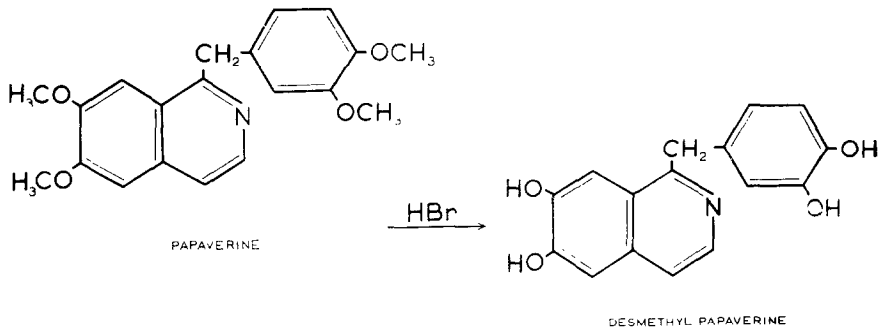

and gassed with 95% O_2 and 5% CO_2. Recording was by an isotonic lever giving a magnification of 10; 5-g tension was applied to the muscle. The strip was left initially for at least 2 hr to permit adequate relaxation before applying the agonist. After it had produced maximal contraction it was washed out and the muscle allowed to relax to the base line before the next addition of agonist. Concentrations of agonists were as follows: epinephrine 0·003–0·3 μM, angiotensin 0·001–0·03 μM, and the potassium ion in concentrations of 20–60 mM. The test compound was added to the bath 5–10 min before testing the response to the agonist. In the few experiments, when the vas deferens of the guinea pig was used, the procedure was similar but a smaller tension, 0·2–0·4 g, was applied to the muscle.

The guinea pig ileal strip suspended in Tyrode's solution at 37° was caused to contract every 4·5 min by exposure to histamine 1×10^{-6} M, carbachol $1{\cdot}5 \times 10^{-6}$ M, or barium cholride $2{\cdot}5 \times 10^{-3}$ M in an automatic biological assay apparatus (Casella Electronics, England). The concentrations of the test drug, expressed as a multiple of that of papaverine, required to cause 50 per cent inhibition of the contraction were estimated.

Cats were anaesthetized i.p. with sodium pentobarbital, 35 mg/kg. Further anaesthetic was administered i.v. as required. Blood pressure was recorded from the left carotid artery with a mercury manometer. Injections were given into the left femoral vein via a polyethylene cannula.

Chemical

Desmethylpapaverine hydrobromide was prepared by refluxing 2 g papaverine hydrochloride in 50 ml of 40% HBr overnight. A white precipitate of desmethylpapaverine hydrobromide was formed which was filtered and washed with acetone (m.p. 250° decomp.[4]). It gave a positive $FeCl_3$ test. The salt was used in subsequent investigations. Papaverine gave a negative $FeCl_3$ test, indicating that there are no detectable phenolic impurities.

Enzymatic

The purified catechol-O-methyl transferase was prepared according to Axelrod and Tomchick[5] except that the purification steps were carried only to the second precipitation with ammonium sulfate. The incubation mixture consisted of 2 ml enzyme solution, 6 ml phosphate buffer (pH 7·8, 0·1 M), 5 mg S-adenosylmethionine iodide, 0·1 ml 1 M $MgCl_2 \cdot 6H_2O$, L-norepinephrine-D-bitartarate in concentrations varying from 6×10^{-4} M–7×10^{-5} M, and desmethylpapaverine in concentrations varying from

1×10^{-4} M–5×10^{-6} M. 4-Methyltropolone (1×10^{-4} M) was also used as an inhibitor. Papaverine (1×10^{-4} M) and kojic acid (5×10^{-5} M) were also assayed as potential inhibitors of COMT. The mixture was made to a total volume of 11 ml by adding water and incubated at 37°. Suitable aliquots were withdrawn at intervals and quenched by the addition of 1 ml of 0·5 M borate buffer (pH 10).

The assay procedure was a modification of the one used by Barac[6] for the quantitative estimation of metanephrine in dog's blood. Each aliquot was extracted with a 25-ml portion of toluene: isoamyl alcohol mixture (3:2).[7] A 20-ml aliquot of the extract was shaken with 3 ml of 0·1 N HCl. Then a 2-ml portion of the acid extract was assayed for normetanephrine as follows. To each 2-ml portion of the acid extract were added 10 ml water, 5 ml 20% Na_2CO_3 solution, and 2 ml of an acetone solution of N,2,6-trichloro-*p*-benzoquinoneimine (1 mg/ml). The resulting blue color was read at 580 mμ after 3 min. No interference by the other components of the incubation mixture was noticeable.

RESULTS

Pharmacological

Isolated tissues. After the responses for three geometrically increasing doses of the agonist had been tested on the aortic strip, the influence of the test drug on the subsequent response to the middle dose of agonist was determined. The results are summarized in Table 1 which lists the number of occasions in which the test drug increased, did not alter, or decreased the response to epinephrine both during contact with the drug and after it had been washed out. The mean magnitude of change, included in parentheses, indicates the number of times the concentration of agonist had to be decreased or increased to produce a similar response to that in the control.

High concentrations of papaverine inhibited the epinephrine contraction; low concentrations moderately potentiated the response, and intermediate concentrations potentiated some strips and inhibited others. Desmethylpapaverine inhibited in only very high concentrations. In low to intermediate concentrations it moderately potentiated responses; at a high intermediate concentration, 3×10^{-5} M, it caused marked potentiation. Kojic acid moderately increased the response to an equal extent at all the concentrations tested. In order to determine whether the greater potentiation obtained with 3×10^{-5} M desmethylpapaverine was specific for epinephrine, its effect on angiotensin and potassium responses was tested. Kojic acid was included for comparison. Preliminary trials showed a straight-line relationship between response and the logarithm of the concentration for each agonist. The slopes with all three agonists (b = 26, 27·8 and 28·9 for epinephrine, angiotensin, and potassium respectively) did not differ significantly from parallelism. The effect of desmethylpapaverine and kojic acid on responses to these three agonists are included in Table 2. While 3×10^{-5} M kojic acid produced an equal potentiation of epinephrine and angiotensin, desmethylpapaverine in a similar concentration potentiated epinephrine responses more than those of angiotensin and potassium.

On the guinea pig ileum, desmethylpapaverine was 70 times and kojic acid 500 times less potent than papaverine as a spasmolytic; no difference was noted against the three different spasmogens. These relative values of spasmolytic potency are approximately similar to those obtained for antagonism of epinephrine contractions on aortic muscle.

TABLE 1. POTENTIATION AND INHIBITION OF EPINEPHRINE ON AORTIC STRIPS

Drug tested	Molar conc.	No. of expts.	No. of trials	Frequency of change*						
				Drug present			After washing out drug			
				+	0	−	+	0	−	Not tested
Papaverine	3×10^{-8}	2	2	1 (2)	1		2 (1·8)			
	3×10^{-7}	2	4	2 (2·5)	1	1 (1·5)		1	1 (1·5)	2
	3×10^{-6}	2	4	1 (2)	1	2 (1·8)	1 (2)	2	1 (1·5)	
	3×10^{-5}	3	3			3 (22)	1 (2)			2
	3×10^{-4}	1	1			1 (20)			1 (10)	
Desmethyl-papaverine	3×10^{-9}	1	1	1 (1·5)			1 (2)			
	3×10^{-8}	3	3	2 (2·5)	1		1 (3)	1		1
	3×10^{-7}	1	1	1 (3)			1 (3)			
	3×10^{-6}	2	2	2 (3)			1 (3)			1
	3×10^{-5}	3	5	4 (16)	1		2 (16)			3
	3×10^{-4}	1	1			inhib.			inhib.	
Kojic acid	3×10^{-8}	2	2	2 (1·8)				2		
	3×10^{-7}	1	1	1 (3)			1 (3)			
	3×10^{-6}	3	5	5 (2·5)			2 (3)	2		1
	3×10^{-5}	2	2	2 (2)						2
	3×10^{-4}	1	1	1 (3)						1

* Increase, +; no change, 0; decrease, −. Mean magnitude of change (in parentheses) indicates the number of times the concentration of epinephrine had to be decreased or increased to produce a similar response to that in the control.

Anaesthetized cat. Desmethylpapaverine, 10 mg/kg, potentiated but did not prolong the pressor effect of epinephrine. The response to 0·3 μg epinephrine/kg after desmethylpapaverine was similar to the response of the cat to 1 μg/kg before desmethylpapaverine (control). Likewise the response to 1 μg/kg after desmethylpapaverine was similar to the response to 3μg/kg in the control before desmethylpapaverine. In another

TABLE 2. DEGREE OF POTENTIATION* BY COMPOUND (10^{-5} M) OF AORTIC STRIP RESPONSES WITH THREE AGONISTS

Compound	Agonist		
	Epinephrine	Angiotensin	Potassium
Desmethylpapaverine	12 ± 4 (3)	1·5 ± 0·3 (6)	1·6 ± 0·14 (4)
Kojic acid	2 (2)	1·3 ± 0·34 (3)	

* Mean ± S.E.M.; number of experiments in parentheses.

experiment it similarly potentiated the pressor response to norepinephrine but not that of angiotensin. This potentiation had disappeared when tested again 15 min later. In a third experiment, the same dose of desmethylpapaverine prolonged but did not potentiate the depressor response to 1 μg isoproterenol/kg. This prolongation showed no recovery.

Enzymatic

Desmethylpapaverine at a concentration of 1×10^{-5} M produced marked inhibition (55%) of COMT, while papaverine at a concentration of 1×10^{-4} M showed no significant inhibition of the enzyme (Fig. 1). Desmethylpapaverine produced 50 per cent inhibition of COMT at a concentration of approximately 8×10^{-6} M. A Lineweaver-Burk plot established the fact that desmethylpapaverine acts competitively.

4-Methyltropolone at a concentration of 1×10^{-4} M produced 76 per cent inhibition under similar conditions, while desmethylpapaverine at the same concentration produced 91 per cent inhibition of COMT (Fig. 1). It has been shown that 4-methyltropolone was a potent inhibitor of COMT,[8] 50 per cent inhibition being produced by 3×10^{-5} M. These results therefore show that desmethylpapaverine is about four times more potent than 4-methyltropolone.

The substrate specificity and cation requirement for enzymatic O-methylation led Senoh *et al.*[9] to propose a chelation mechanism to account for the methyl group transfer reaction from adenosylmethionine to the *m*-phenolic group of substrates. Since tropolones form stable chelates with divalent metal ions, a property which is shared by catechol rings, and since the tropolone ring is isosteric with the catechol ring, a similar chelated complex formation with COMT was suggested.[10]

Kojic acid, which chelates certain metal ions but is not isosteric with the catechol ring, produced no inhibition of COMT at a concentration of 5×10^{-5} M.

DISCUSSION

Low and intermediate concentrations (3×10^{-9} M–3×10^{-6} M) of desmethylpapaverine produced moderate potentiation of epinephrine responseses similar to that achieved with low concentrations (3×10^{-8} M–3×10^{-7} M) of papaverine and

all tested concentrations (3×10^{-8} M–3×10^{-4} M) of kojic acid. A higher concentration of desmethylpapaverine (3×10^{-5} M), unlike that of papaverine or kojic acid, markedly potentiated epinephrine but not angiotensin- or potassium-induced responses. Very high concentrations (3×10^{-4} M) of desmethylpapaverine masked this extreme potentiation owing to a spasmolytic effect. The moderate potentiation,

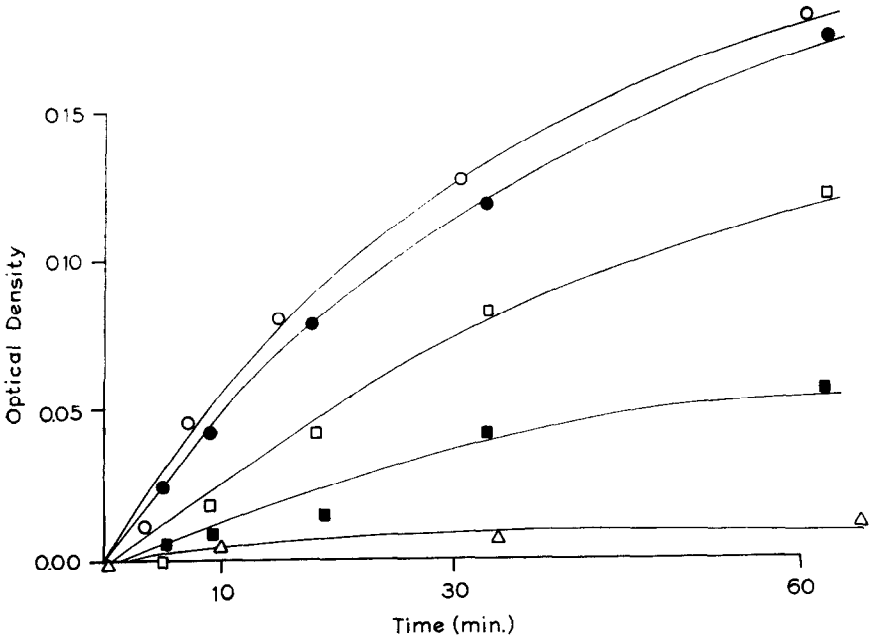

FIG. 1. Rate curves for the COMT-catalyzed methylation of norepinephrine: ○, control, L-norepinephrine-D-bitartarate 6×10^{-4} M; ●, L-norepinephrine-D-bitartarate 6×10^{-4} M + papaverine 1×10^{-4} M; □, L-norepinephrine-D-bitartarate 6×10^{-4} M + desmethylpapaverine 1×10^{-5} M; ■, L-norepinephrine-D-bitartarate 6×10^{-4} M + 4-methyltropolone 1×10^{-4} M; △, L-norepinephrine-D-bitartarate 6×10^{-4} M + desmethylpapaverine 1×10^{-4} M.

produced by desmethylpapaverine, kojic acid, and papaverine on responses by epinephrine, angiotensin, and potassium appears to be due to a nonspecific effect on the muscle tissue. The marked potentiation which was produced by 3×10^{-5} M desmethylpapaverine was specific for epinephtine. This suggests that the marked potentiation of the epinephrine-induced contraction may be due to inhibition of COMT. This is substantiated by the finding that desmethylpapaverine at the concentration which produced marked potentiation of epinephrine on the aortic strip, i.e. 3×10^{-5} M, caused approximately 80 per cent inhibition of the anzyme. At one tenth this concentration it inhibited the enzyme only about 10 per cent and had no specific potentiating effect on epinephrine. At 3×10^{-4} M it inhibited the enzyme completely but, owing to the spasmolytic effect at this concentration, the potentiating effect was completely masked on the aortic strip. The potentiation of epinephrine and norepinephrine pressor responses, but not those of angiotensin, and the prolongation of the depressor response of isoproterenol by a concentration which produced marked enzyme inhibition *in vitro* suggests that these effects may also be due to inhibition of COMT.

Demethylation of papaverine reduced its spasmolytic activity about 70 times; alkylation of the hydroxyl groups is evidently a prerequisite for this pharmacological property.[11] Kojic acid did not inhibit COMT and did not cause a specific potentiation of epinephrine. It also had no spasmolytic activity. The potent inhibition of COMT by desmethylpapaverine is possibly related to the double catechol centres available for this effect. Also, it is quite probable that desmethylpapaverine is a substrate for COMT.

The initial moderate potentiating effect of papaverine in low concentrations remains ambiguous. It is very probably due to a nonspecific effect.

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