AUGMENTATION OF PHARMACOLOGICAL PROPERTIES OF CATECHOLAMINES BY O-METHYL TRANSFERASE INHIBITORS

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Maclagan and Wilkinson (1954) first showed that phenolic hydroxyl groups could be methylated in man. Subsequently, Axelrod (1957) demonstrated methylation at the 3-hydroxy position of epinephrine and norepinephrine *in vitro* and *in vivo*, and later (1958) that the amines undergo oxidative deamination only after they have been methylated. The *m*-O-methylnorepinephrine (normetanephrine) formed by methylation possesses only 1/500 of the activity of norepinephrine, suggesting that this metabolic process is the means of inactivation of these amines (Evarts *et al.*, 1958).

An O-methyl transferase (OMT) inhibitor would prolong the activity of epinephrine and many substances have been reported to do this; Bacq, for instance (1936a, b), described augmentation of epinephrine effects by polyphenols. As polyphenols of the catechol type are also methylated at the 3-hydroxy position (Booth *et al.*, 1955; Archer *et al.*, 1960) it occurred to us that the augmentation observed by Bacq could result from competitive affinity for the same enzyme system (Wylie *et al.*, 1959).[1]

METHODS. *In vitro*. The O-methylation of epinephrine was estimated by the procedure of Axelrod and Tomchick (1958). The procedure depends on the incubation of 1.5 μmol of *l*-epinephrine-D-bitartrate, 50 μmol magnesium chloride, 1.5 μmol of S-adenosyl-methionine (Cal. Found. for Biochem. Res.), 50 μmol phosphate buffer (pH 7.8), enzyme and water to 5.0 ml at 37°C. Inhibitors were preincubated in the system prior to the addition of epinephrine and S-adenosyl-methionine for 10 minutes. At the end of the incubation period of 30 or 60 minutes, the reaction in a 1.0-ml aliquot was stopped by the addition of 0.5 ml of borate buffer (pH 10.0). The formed metanephrine was extracted into 20 volumes of ethylene dichloride-isoamyl alcohol (2%) and extracted, in turn, into 2.0 ml HCl (0.1 N). The metanephrine was estimated fluorimetrically[2] at 335 mμ after activation at 285 mμ.

In vivo. Spinal cats were prepared by sectioning the cord at C2 and destroying the brain by means of a rod introduced through the foramen magnum. Following destruction of the brain the animals were allowed to recover from the anesthesia. The blood pressure was recorded by catheterization of the carotid artery. One hour following destruction of the brain, graded submaximal doses (0.5 to 4.0 μg) of epinephrine and norepinephrine were administered intravenously and an appropriate submaximal dose was repeated until it produced equal pressor responses consecutively. The OMT inhibitors were given either before or with the amines and the pressor effects compared.

Supramaximal stimuli were applied to the preganglionic sympathetic nerve trunk so that equal contractions of the nictitating membrane were obtained. Following intravenous administration of the inhibitor, the same stimulus was applied and the height and duration of the contractions compared with the controls.

White male rats of 80 to 100 g were used for intravenous toxicity determinations. The animals were observed for 24 hours for death. The calculations of the LD50 were made by the Miller and Tainter method (1944).

l-Epinephrine bitartrate and *l*-norepinephrine bitartrate monohydrate were used in all experiments and the doses reported are of the base; the solutions contained 0.01% ascorbic acid and were kept cold. Fresh solutions were made every 4 hours.

RESULTS. Under *in vitro* conditions we found that a series of polyphenols markedly inhibited the rate of methylation of epinephrine (table 1), and there was approximately a 16-fold difference in their activities. Cocaine, which powerfully augments epinephrine effects on smooth muscle

[1] Wylie, D. W., Archer, S. and Arnold, A.: Pharmacologist 1: no. 2, p. 54, 1959.

[2] Aminco-Bowman spectrophotofluorometer.

preparations, did not, however, affect methylation.

Adrenalone (ω-[N-methylamino]-3,4 dihydroxyacetophenone) and arterenone (ω-amino-3,4 dihydroxyacetophenone) are inhibitors of OMT (Udenfriend, personal communication) and it is interesting to note that under *in vitro* conditions these ketone analogs of epinephrine and norepinephrine had a much greater affinity for the enzyme than did their hydroxy counterparts. While catechol was indicated by Axelrod and Tomchick (1958) to be methylated somewhat more readily than epinephrine, it may be seen from table 1 that the trihydroxy phenols, pyrogallol and gallic acid, appeared to be considerably more sensitive to methylation than was epinephrine, since they markedly interfered with the formation of metanephrine.

TABLE 1

OMT inhibitory activities of polyphenols using 3×10^{-4} M epinephrine as substrate

Inhibitor	Epinephrine I50 Molar Conc. Estimated	Relative Activity Catechol = 1
Catechol	5×10^{-4}	1
Adrenalone	5×10^{-5}	10
Arterenone	6×10^{-5}	8
Pyrogallol	3×10^{-5}	16
Gallic acid	5×10^{-5}	10
Cocaine	Inactive	—

Luduena *et al.* (1959) reported that intravenous epinephrine, in doses of 50 to 100 μg/kg, caused pulmonary edema and death in rats. The data in table 2 show that simultaneous administration of polyphenols augmented this toxicity considerably.

Intravenous norepinephrine also caused pulmonary edema and death in rats, but it was not as toxic as epinephrine. Pyrogallol, at twice the dose which augmented the toxicity of epinephrine, did not alter the toxicity of norepinephrine; catechol, adrenalone and arterenone showed potentiating activity but less than was seen with epinephrine (table 2). Thus the differences in LD50's of the two amines and their differing degree of augmentation by OMT inhibitors do not appear to be related to differences in their mechanisms of action in causing death. Furthermore,

TABLE 2

*Effect of polyphenols on intravenous toxicity of epinephrine and norepinephrine in rats**

Inhibitor	Dose Inhibitor mg/kg i.v.	Effect of Inhibitor on Toxicity of Catecholamines i.v. LD50 ± S.E. μg/kg		i.v. LD50 ± S.E. Inhibitor mg/kg
		Epinephrine	Norepinephrine	
—	—	45.0 ± 7.5	95.0 ± 12.3	—
Catechol	5.0	27.5 ± 4.1	—	95.0 ± 15.4
	10.0	19.5 ± 2.1	98.4 ± 16.0	
	20.0	19.5 ± 2.1	48.0 ± 11.2	
Adrenalone	0.5	21.0 ± 7.0	63.0 ± 7.0	42.5 ± 5.4
Arterenone	0.5	22.0 ± 6.2	63.0 ± 7.2	100.0 ± 23.0
Pyrogallol	20.0	46.0 ± 10.3	—	565 ± 59.0
	40.0	22.0 ± 3.4	—	
	80.0	—	100.0 ± 3.2	
Gallic acid	400.0	25.5 ± 7.7	—	>2000
Cocaine	0.1	29.0 ± 6.5	—	12.5 ± 1.1
	0.2	18.8 ± 5.8	—	

* Twenty rats were used at each dose level and a minimum of three dose levels were tested for each agent or combination of agents.

the data in table 3 show that the toxicity of epinephrine, when augmented by an OMT inhibitor, was still adrenergic in mechanism, as the same dose of chlorpromazine protected the animals from epinephrine plus inhibitor as from epinephrine alone. These results should be expected as the same amount of epinephrine was administered when given alone and when given with inhibitor; doubling the dose of epinephrine made it necessary to increase the dose of adrenolytic.

The relative activities of the polyphenols as inhibitors of the methylation of epinephrine were different in the rat experiments from those obtained in the *in vitro* studies. Adrenalone and arterenone possess $^1/_{50}$ to $^1/_{100}$ of the activity of their respective hydroxylated amines, so that augmentation may be partially the effects of summation of sympathomimetic effects. The adrenergic action of the ketones was further confirmed by the finding that they caused death in a manner similar to epinephrine and that rats could be protected from 3 × LD50 dose of adrenalone by the adrenolytic chlorpromazine in a manner similar to the protection from epinephrine toxicity by chlorpromazine. The doses of adrenalone and arterenone used to augment the toxicity of epinephrine were well below their respective LD50's, and, thus, in view of the enzyme results, their action would appear to be synergistic rather than additive.

TABLE 3

Adrenolytic activity of chlorpromazine against i.v. epinephrine alone and with catechol (OMT inhibitor)

Dose Epinephrine μg kg i.v.	Dose of Catechol mg kg i.v.	Adrenolytic Potency* Chlorpromazine ED50 ± S.E., μg kg
200	—	40.5 ± 3.8
400	—	108.0 ± 23.5
200	20	42.5 ± 7.3

* To determine adrenolytic potency the drugs were administered simultaneously. Four doses of chlorpromazine were used in each test, 10 rats per dose (Luduena, 1959).

The other polyphenols alone, unlike adrenalone and arterenone, did not cause epinephrine-like effects and the animals were not protected from their various actions by adrenolytic agents. Catechol when given alone caused marked twitching, hyperreflexia and tonic convulsions followed by death. The appearance was identical to that produced by strychnine. Twitching and hyperreflexia were also noted in the spinal cat, suggesting action at the spinal level. These responses were unaffected by chlorpromazine given either simultaneously with the catechol at 16 mg/kg i.v. or 40 minutes before the catechol at 64 mg/kg s.c. Chlorpromazine is centrally active and has adrenolytic properties; it was therefore unlikely that the central effects of catechol described above were due to an accumulation of

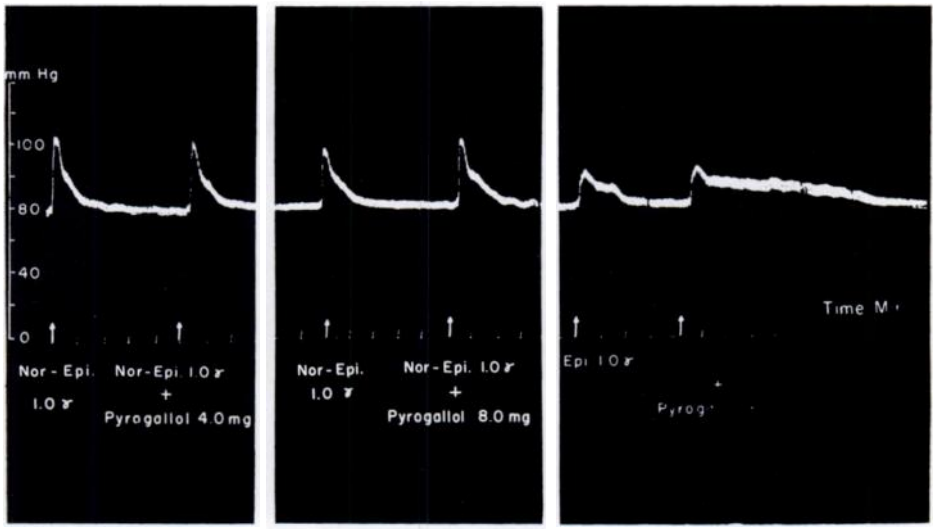

FIG. 1. Effects of catechol and pyrogallol on the duration of pressor effects of intravenous epinephrine.

sympathomimetic amines due to inhibition of OMT.

In the spinal cat preparation, we found that all of the polyphenols in table 1 prolonged the pressor effects of epinephrine (fig. 1) in agreement with the data presented by Bacq (1936a).

Pyrogallol and catechol, when given simultaneously with epinephrine, caused increased duration of action. Larger doses (5.0 mg) had pressor effects but if the administration of the epinephrine was delayed until the blood pressure had returned to normal the effects of the epinephrine were still prolonged.

All of the OMT inhibitors tested caused some pressor effects when given alone, as will be discussed later, but the effects were small or not apparent at the dose levels used to augment epinephrine activity. However, adrenalone and arterenone had sufficient pressor activity to make interpretation of the results difficult.

Augmentation of the pressor effects of norepinephrine was not readily produced by pyrogallol and catechol. A total of 7 cats have been used and the results indicate that in no instance was it possible to prolong the pressor effects of norepinephrine in as effective a manner as was possible with epinephrine (fig. 2).

Large doses of pyrogallol increased the duration of contraction of the cat nictitating membrane in response to supramaximal stimulation of the preganglionic sympathetic nerve (fig. 3). This has been repeated in 12 cats. In every case the contraction following the intravenous administration of 160 mg pyrogallol was of longer

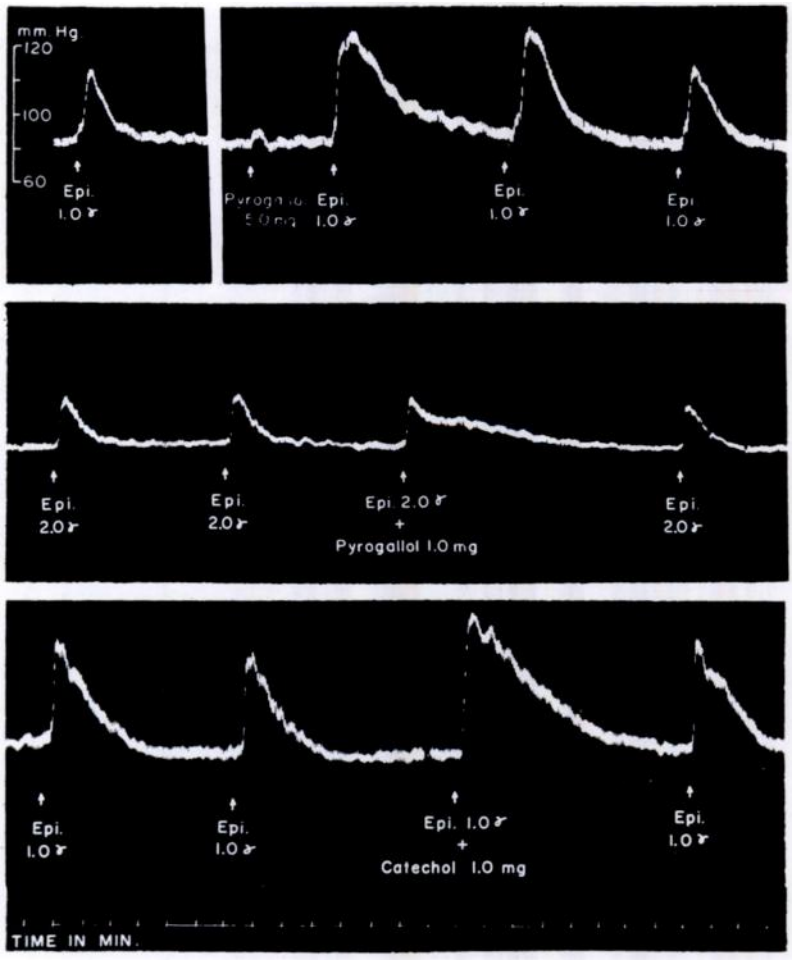

FIG. 2. Augmentation of epinephrine and norepinephrine by pyrogallol in the same cat.

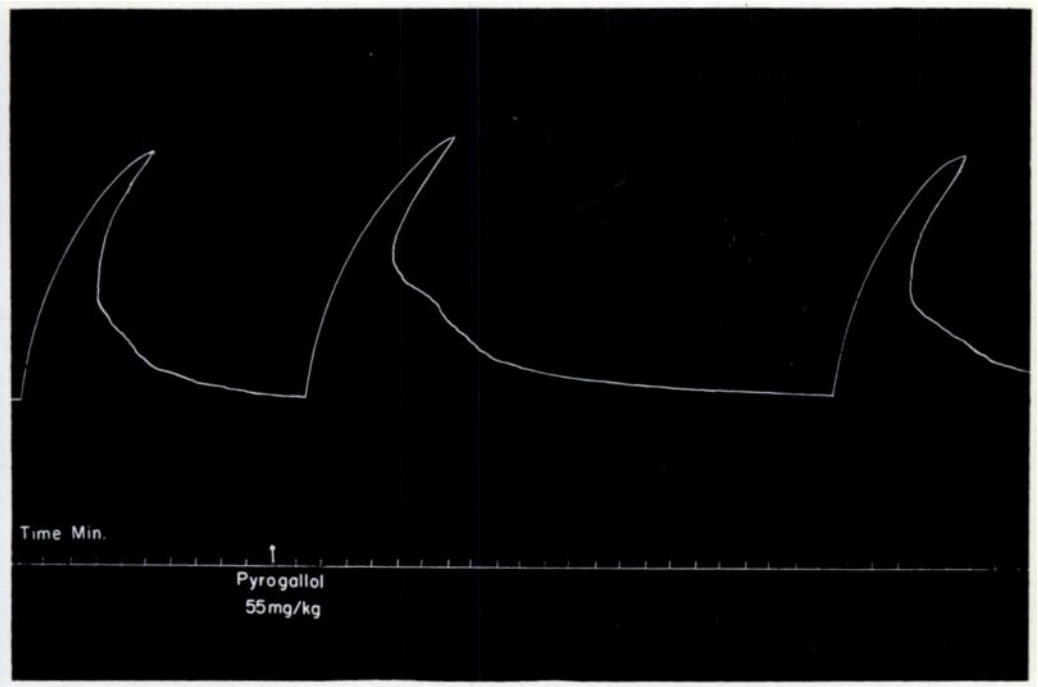

FIG. 3. Effect of pyrogallol on the contraction of the nictitating membrane following stimulation of the preganglionic sympathetic nerve. (Duration of stimulation 30 secs. Frequency 24/sec V = 5.)

duration than the controls, and for 30 minutes following the medication subsequent responses to stimulation were also prolonged. After the second administration of pyrogallol, however, augmentation of the contraction was not so marked.

Burn (1958) showed that the tonus of the blood vessels was probably maintained by constant production of sympathomimetic amines at the nerve endings. Maintenance of a sufficient blood level of an OMT inhibitor, therefore, should cause some pressor effect and it can be seen from fig. 1 that a single injection of pyrogallol had slight pressor effects. Following repeated single administrations of pyrogallol, the general level of the blood pressure rose at first followed by tachyphylaxis to further administrations.

DISCUSSION. Various polyphenols are methylated at the 3-hydroxy position in the same way as epinephrine, and it is probable that the polyphenols reported here have reduced methylation of epinephrine by acting as competitive substrates for the enzyme system.

The data we have obtained suggest that the augmentation of epinephrine effects by polyphenols is probably due to an increased life-span of the epinephrine because of this reduced rate of methylation.

The effects of circulating norepinephrine in rats and cats were not augmented by pyrogallol or catechol as effectively as the epinephrine responses, while under *in vitro* conditions these polyphenols inhibited methylation of both amines.

A possible explanation for this difference between epinephrine and norepinephrine could be that norepinephrine has a greater affinity for O-methyl transferase than does epinephrine and was thus more difficult to displace by a competitive inhibitor, especially since both catecholamines and the polyphenols have very short durations of action. However, under *in vitro* conditions no differences in affinities of the amines for the enzyme system could be detected, the Km for epinephrine was found to be 2.3×10^{-4} M and that for norepinephrine was 3.5×10^{-4} M.

All of the blood pressure experiments involved intravenously administered catecholamines. However, our experiments using the cat nictitating membrane preparation, showed that OMT inhibitors were also able to delay methylation of

endogenously released catecholamine, which at this site is predominantly norepinephrine.

Axelrod (1959) found that methylation of epinephrine *in vivo* occurred in two phases which differed in velocity; there was a phase of rapid methylation in which more than half (60 to 70%) of the injected amine was methylated within 10 minutes, the remaining methylation being extended over 3 or more hours. Axelrod theorized from his data that, although O-methylation started immediately, some of the epinephrine was also bound rapidly onto tissues from which it was subsequently released and methylated. Such an immediate dual mechanism of methylation and binding would help account for the findings that the physiological response to injected epinephrine is completed even when unchanged epinephrine is still detectable in the animal. Our data suggested that inasmuch as there is no difference in the affinities of the two amines for the enzyme, the difficulty in augmenting circulating norepinephrine by pyrogallol could possibly be accounted for by the fact that norepinephrine may be more readily bound and thus physiologically inactivated than is epinephrine. Axelrod found independently (personal communication) that in the case of norepinephrine less than half was immediately methylated, the major portion being bound and slowly liberated and methylated. If binding is also a method of physiological inactivation, the differences in affinities of the two amines for binding sites might explain the differences obtained in our experiments. It could be further postulated that adrenalone and arterenone might owe their greater ability to potentiate *both* amines to the fact that they chemically resemble the amines and may compete with them for both the enzyme and binding sites.

Inhibition of O-methyl transferase is obviously not the only means whereby epinephrine can be augmented, as cocaine, for instance, has the latter property to a high degree but is inactive as an inhibitor of this enzyme system.

SUMMARY

A series of polyphenols have been found to reduce methylation of epinephrine and norepinephrine by O-methyl transferase *in vitro*, probably by acting as competitive substrates.

These substances augmented the toxicity of intravenous epinephrine in the rat, the augmented toxicity being adrenergic in nature and probably due to an increase in the life-span of epinephrine. The effects with norepinephrine were less pronounced.

The inhibitors prolonged the duration of the pressor effects of epinephrine in the spinal cat, but were less active in prolonging the effect of norepinephrine.

Pyrogallol, when given repeatedly, caused a rise in blood pressure, but this was soon followed by what appeared to be tachyphylaxis.

Pyrogallol prolonged the duration of action of endogenously released catecholamines at the nictitating membrane.

Possible explanations for the difference between epinephrine and norepinephrine are discussed.

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