Interaction Catecholamines-Pyrogallol on Rabbit Aortic Strips

The action of COMT on the catecholamines and their derivatives can be inhibited both in vivo and in vitro by pyrogallol, as well as by other catechols. These are competitive inhibitors and are themselves substrates for the enzyme; therefore the effects of catecholamines are augmented by pyrogallol[1,2] because the velocity of methylation is reduced[3]. However, the inhibition of COMT in vivo has little effect on the actions of noradrenaline but potentiates the actions of injected adrenaline. A possible explanation for this difference could be that noradrenaline has a greater affinity for COMT than has adrenaline and was more difficult to displace by a competitive inhibitor[4].

The object of this paper is to compare the potentiation of the effects of adrenaline and noradrenaline by pyrogallol on the aortic strips of the rabbit.

Methods. Spiral strips of rabbit thoracic aorta were prepared as described FURCHGOTT and BHADRAKOM[5]. The solution was Krebs-bicarbonate solution[6] containing 0.01*M* glucose. Through this solution 95% O_2, 5% CO_2 was bubbled giving a pH of 7.4. The bath's temperature was maintained at 37.5 °C. The recording was made with a Grass Poligraph Model 79-2 with a transducer force displacement FT 03. The initial tension of the strips was 4 g. The following drugs were used: l-noradrenaline bitartrate; l-adrenaline bitartrate; acetylcholine bromide and pyrogallol.

In 6 aortic strips from different rabbits, dose-response curves with adrenaline and noradrenaline were obtained under both control conditions and in the presence of pyrogallol.

Two dose-response control curves were also obtaneid with adrenaline and noradrenaline in the same aortic strip, in each of 4 strips of different rabbits to discard a possible spontaneous potentiation in the second curve. Another dose-response curves were obtained in 5 experiments with an agent no affected by COMT, such as acetylcholine in both control conditions and in the presence of pyrogallol to discard a possible unspecific effect of this compound.

The strips used were all from different aortas. All the doses were cumulative and all concentration figures refer to the concentrations of the drugs added into the organ-chamber of 10 ml. The recovery time, after washing, between the dose-response curves in the same strip was about 2 h. The curves in the presence of pyrogallol were began 1 min after the addition of this compound. Concentration-activity curves based on mean values and standard errors were carried out.

Results. The concentration-activity curves show that the effect of adrenaline is clearly increased (Figure 1 A) while the effect of noradrenaline only exerts a light potentiation under the same conditions (Figure 1 B). This potentiation of both catecholamines only affects the first 3 or 4 doses. The concentration-activity curves with adrenaline and noradrenaline do not show significant differences when repeated twice in control conditions (Figure 2 A). The concentration-activity curve obtained with acetylcholine shows that the dose-response curves with this agent were unchanged when repeated in the presence of pyrogallol (Figure 2 B).

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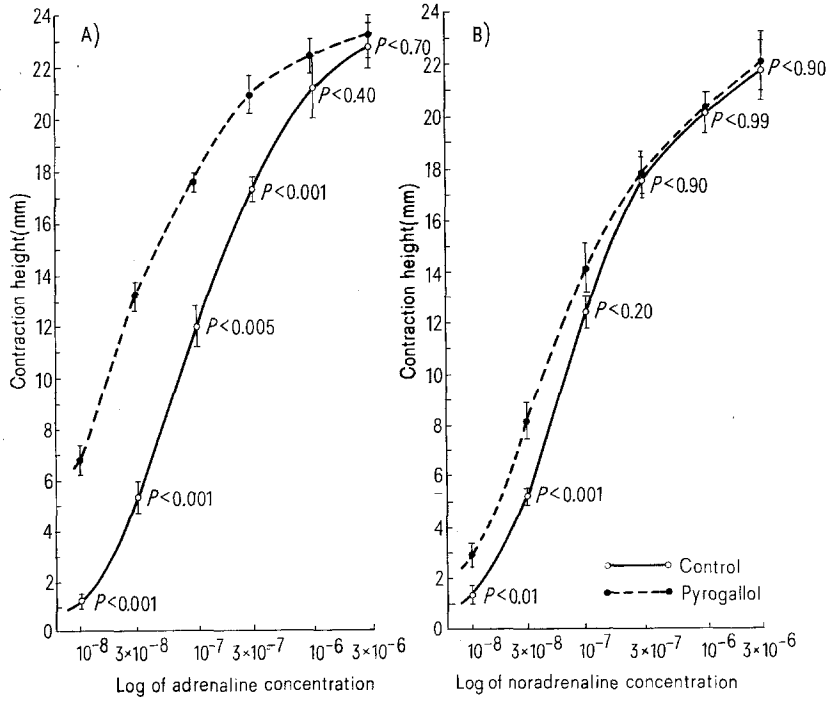

Fig. 1. Concentration-activity curves obtained with adrenaline (A) and noradrenaline (B) in both control conditions and in presence of pyrogallol (10^{-5}). All drug concentrations are expressed in g/ml. Each point represents the mean of 6 experiments ± SEM.

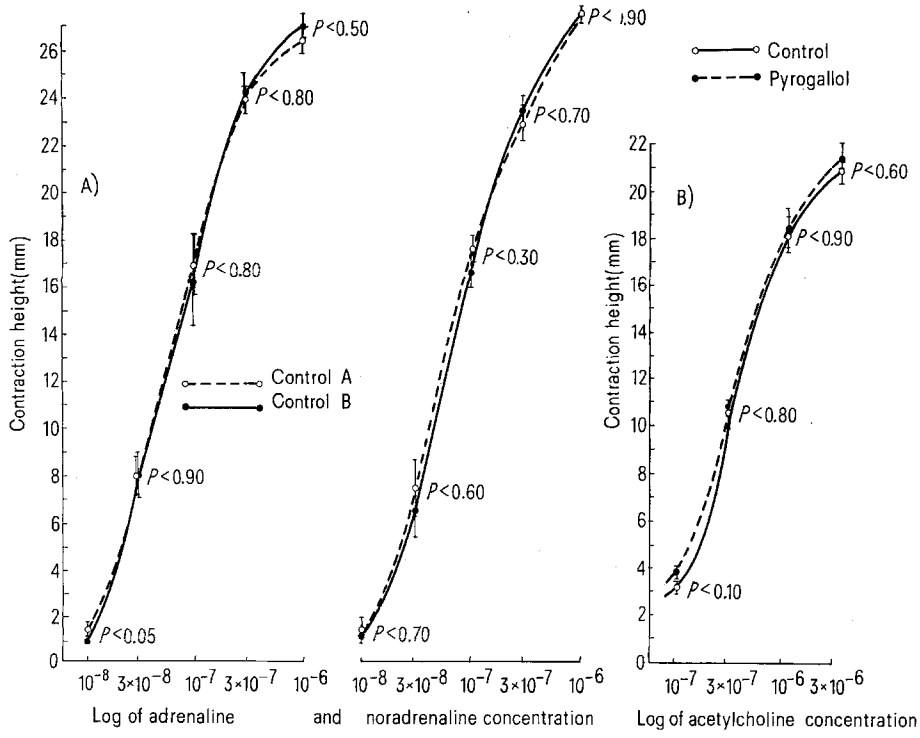

Fig. 2. A) Concentration-activity curves obtained with adrenaline and noradrenaline of 2 successive dose-response control curves. Each point represents the mean of 4 experiments ± SEM. B) Concentration activity curves obtained with acetylcholine in both control conditions and in presence of pyrogallol (10^{-5}). Each point represents the mean of 5 experiments ± SEM. All drug concentrations are expressed in g/ml.

Discussion. WYLIE, ARCHER and ARNOLD[3] suggest that the increase of adrenaline effects by polyphenols is probably due to an increased life-span of the adrenaline because of a reduced rate of methylation. This reduced rate explains the difference between adrenaline and noradrenaline, because noradrenaline has a greater affinity for COMT than does adrenaline and is more difficult to displace by a competitive inhibitor, while under in vitro conditions these polyphenols inhibited methylation of both amines.

On the other hand, AXELROD[7] found that the methylation of adrenaline in vivo occurred in 2 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 min, the remaining methylation being extended over 3 or more h. In the case of noradrenaline, less than half was immediately methylated and the major portion was slowly liberated and methylated.

This difference in affinity of the 2 amines for binding sites might explain the differences obtained in our experiments on rabbit aortic strips, since the inhibition of COMT prevents the methylation of the major portion of adrenaline (60 to 70%) in the first phase, which is responsible for potentiation. The absence of potentiation in the last doses of the curve might be explained by the fugacity of action of pyrogallol. Another possible explanation for this difference could be that the difference of uptake between adrenaline and noradrenaline into adrenergic nervous endings, influences the difference of potentiation of the two amines after inhibition by polyphenols, since COMT is an extraneuronal enzyme.

A possible significative spontaneous potentiation by an increase of sensitivity of the aortic strips in 2 successive dose-response curves, is discarded. The absence of potentiation of responses to acetylcholine, which is not affected by COMT, supports the specificity of this potentiation for substances metabolized by COMT.

Resumen. El Pyrogallol potencia claramente los efectos de adrenalina y debilmente los de noradrenalina en la preparación de tiras aisladas de aorta de conejo. Los efectos de acetilcolina no son potenciados en la misma preparación.

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Phallolysin, ein hochmolekulares Toxin aus *Amanita phalloides*

Aus dem grünen Knollenblätterpilz *Amanita phalloides* (Vaill. ex Fr.) Secr. konnten wir durch wässrige Extraktion Phallolysin, ein hochmolekulares, hämolytisch wirkendes Toxin gewinnen. Es war alkohollabil; bei der methanolischen Extraktion zur Isolierung der bekannten Knollenblätterpilzgifte – Phalloidin, Amanitine, weitere toxische Cyclopeptide – (WIELAND[1]) wurde es zerstört. Sein Anteil an der Gesamttoxizität grüner Knollenblätterpilze (für Mäuse i.p.) betrug in unserem Ausgangsmaterial 40–75% (Tabelle).