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The potentiation of rat uterine inhibitory responses to noradrenaline by theophylline and nitroglycerine

B. LEVY AND B. E. WILKENFELD

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas, U.S.A.

1. Dose response curves for noradrenaline were determined before, during and after pretreatment with a variety of agents. The inhibitory response to noradrenaline on the isolated rat uterus was measured according to its ability to inhibit an acetylcholine induced contraction.

2. Theophylline, an agent that inhibits phosphodiesterase and increases cyclic AMP levels in the rat, potentiated the response to noradrenaline.

3. Pretreatment with cocaine had no significant effect on the potentiation of the noradrenaline response by theophylline.

4. Theophylline potentiated the inhibitory response to nitroglycerine.

5. Pretreatment with nitroglycerine also potentiated the inhibitory response to noradrenaline.

6. Theophylline appears to produce a potentiation of the inhibitory effect of noradrenaline on the rat uterus by means of a non-specific mechanism.

Adenosine 3′,5′-monophosphate (cyclic AMP) has been suggested as the intracellular mediator for many of the responses to catecholamines (Sutherland & Rall, 1960). The intracellular level of cyclic AMP is controlled by the two enzyme systems adenyl cyclase and a phosphodiesterase. Adenyl cyclase catalyses the conversion of ATP to cyclic AMP, and the phosphodiesterase catalyses the breakdown of cyclic AMP to the inactive 5′-AMP (Butcher & Sutherland, 1962). Adenyl cyclase is activated by catecholamines. The sequence of events in this system is initiated by the activation of adenyl cyclase by an agent such as noradrenaline. This results in an increase in cyclic AMP which in turn activates a series of phosphorylase activating reactions resulting in the characteristic metabolic or mechanical response to noradrenaline. Specific examples of these end responses are the glycogenolytic and positive inotropic responses to noradrenaline. The phosphodiesterase involved in the breakdown of cyclic AMP is inhibited by the methylxanthines (Butcher & Sutherland, 1962). Theophylline is therefore able to increase cyclic AMP levels by preventing its destruction.

Robison, Butcher & Sutherland (1967) suggested that adenyl cyclase is the beta receptor in perhaps all tissues. If adenyl cyclase is the beta receptor then the

responses to agents such as the catecholamines, which stimulate adenyl cyclase, should be potentiated by drugs such as theophylline which inhibit phosphodiesterase activity (Sutherland & Robison, 1966 ; Sutherland, Robison & Butcher, 1968). Rall & West (1962) reported that the positive inotropic response to noradrenaline by electrically driven isolated rabbit atria was increased by theophylline. They concluded that the positive inotropic response to noradrenaline was mediated by cyclic AMP. Lundholm, Mohme-Lundholm & Svedmyr (1966) reported that the methylxanthines potentiate the relaxant effect of catecholamines in intestinal smooth muscle.

The purpose of this study was to determine the effects of theophylline on the inhibitory response to noradrenaline in the isolated rat uterus. The rat uterus possesses primarily only beta receptors subserving inhibition or relaxation (Levy & Tozzi, 1963 ; Patterson, 1965). Dobbs & Robison (1968) have reported that isoproterenol inhibits rat uterine motility and concomitantly causes an increase in intracellular level of cyclic AMP and that both these effects are increased by theophylline. They concluded that the beta receptor was closely related to the adenyl cyclase system.

Nitroglycerine was used in this study both as an agonist and as a possible potentiating agent of the noradrenaline inhibitory response. Brody & Diamond (1967) have reported that nitroglycerine inhibits rat uterine motility without affecting the adenyl cyclase system. Moreover, nitroglycerine has long been characterized as an agent which resembles theophylline in producing a direct depressant effect on smooth muscle.

Methods

Female rats of the Sprague–Dawley strain, weighing 140–160 g were used throughout. All animals were pretreated with 0.1 mg of stilboestrol injected subcutaneously 24 hr before they were used. Rats were killed by a blow on the head. The two horns of the uterus were dissected out and were suspended in 10 ml. organ baths at a constant temperature of 24° C. De Jalon solution (g/100 ml.: NaCl 0.9, KCl 0.042, $CaCl_2$ 0.006, glucose 0.05 and $NaHCO_3$ 0.05) bubbled with 95% oxygen and 5% carbon dioxide, was used in all studies. Submaximal uterine contractions were evoked by the addition of 1.1×10^{-5} moles of acetylcholine chloride to the bath. This dose of acetylcholine was allowed to act for 30 sec and was then washed out. The muscle was washed three times between additions of the drug. This acetylcholine-induced contraction was obtained at 4 min intervals throughout all the experiments performed. Contractions were recorded isometrically by means of a force displacement transducer (Grass model Ft-3). Recordings were made with a multichannel polygraph (Grass model 5 D). The agonists were allowed to act for 3 min before the addition of acetylcholine. Dose response curves for all agonists were determined according to their ability to reduce the acetylcholine-induced contractile response by 50% or more. Unless otherwise specified, the following drug schedule was used throughout. After obtaining a consistent contractile response to acetylcholine, a control dose response curve to the agonist was obtained. Each dose of the agonist was washed out after its 3 min incubation period and each succeeding dose was added in this manner until a dose of the agonist produced an inhibition of the acetylcholine induced contractile response of more than 50%. Following this, the agonist was washed out and after the acetylcholine response was

restored to normal, the test substances theophylline or nitroglycerine were added to the perfusion fluid. These agents were allowed to act for approximately 28 min. During this time acetylcholine was added regularly at 4 min intervals. Dose response curves to the agonists were again determined. All drugs were then removed from the perfusion fluid and after the acetylcholine response returned to control levels another control dose response curve to the agonist was obtained.

Drugs. (−)-Noradrenaline bitartrate was prepared daily from a stock solution with a concentration of 10^{-2}M and containing 10^{-5}M EDTA as a preservative. Acetylcholine was prepared daily from a stock solution of 1:500. Theophylline was used in the form of aminophylline and all doses are expressed in terms of its theophylline content. Cocaine hydrochloride was also used.

The statistical analysis, involving calculation of pD_2 values, was made according to the methods described by Schild (1947). All dose response curves were plotted using the mean responses obtained from eight rat uterine segments for each type of drug pretreatment.

Results

Effect of theophylline on the inhibition response to noradrenaline

Theophylline was capable of reducing the acetylcholine-induced rat uterine contraction by itself if given in sufficient amounts. After a series of preliminary studies we determined the maximum dose of theophylline that could be given and would still produce no reduction in the acetylcholine response. A dose of 5×10^{-5}M theophylline was selected and was used in all studies. Increasing this dose to 1.5×10^{-4}M quite often reduced the acetylcholine response itself. Noradrenaline dose response curves were obtained before, during and after theophylline treatment. Molar doses of noradrenaline in the range of 10^{-9}–10^{-4}M were used. Treatment with theophylline resulted in a significant increase in the pD_2 value for noradrenaline as is shown in Table 1. The control pD_2 value determined after removal of the theophylline was not significantly different from the first control pD_2 value. As is shown in Fig. 1, theophylline treatment resulted in a shift of the dose response curve of noradrenaline to the left. After removal of the theophylline, the dose response curve shifted back to the right. Theophylline produced a 4.9-fold increase in potency of noradrenaline in this test system.

TABLE 1. *Effect of theophylline inhibitory response to noradrenaline*

First control	Theophylline-treated (5×10^{-5}M)	Last control
pD_2	pD_2	pD_2
6·15	6·30	6·0
5·66	6·60	6·13
5·45	6·00	5·25
6·07	6·00	5·61
5·50	6·03	5·68
5·44	6·36	5·87
6·07	7·35	6·16
6·64	7·80	7·04
Mean 5·87	6·56	5·96
±S.E. 0·15	0·238	0·187
P	*<0·05	>0·05

*Differs from control (paired *t* test).

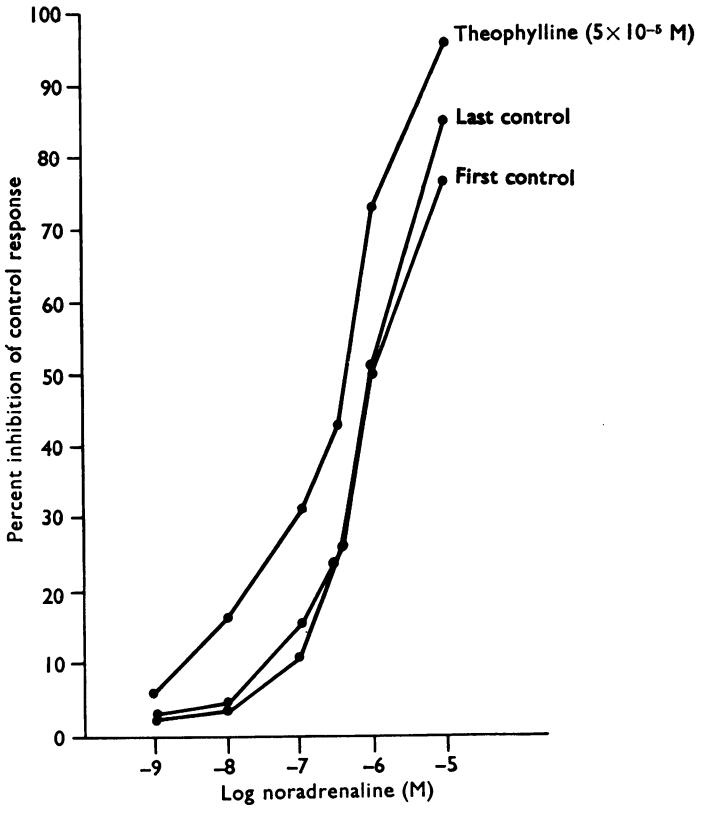

FIG. 1. Effect of theophylline treatment (5×10^{-5}M) on the noradrenaline inhibition of the acetylcholine-induced contractions (1.1×10^{-5}M) in the isolated rat uterus. Log doses of noradrenaline were plotted against the per cent reduction in the acetylcholine-induced contraction. The first control dose response curve was determined before the addition of theophylline. The last control dose response curve was determined after removal of the theophylline. Each point represents the mean of eight experiments.

TABLE 2. *Effect of cocaine plus theophylline on the response to noradrenaline*

First control (cocaine $2\cdot9\times10^{-5}$M) pD_2	Theophylline-treated (5×10^{-5}M) plus cocaine ($2\cdot9\times10^{-5}$M) pD_2	Last control (cocaine $2\cdot9\times10^{-5}$M) pD_2
5·90	6·08	6·08
5·85	6·25	5·90
6·30	6·95	6·33
6·85	8·0	6·90
5·80	6·05	5·55
5·80	6·35	5·70
5·78	6·35	5·68
6·30	6·75	6·43
Mean 6·07	6·60	6·07
±S.E. 0·13	0·23	0·16
P	*<0·05	>0·05

* Differs from control (paired *t* test).

Effect of cocaine and theophylline on the inhibitory response to noradrenaline

The purpose of this procedure was to determine the effects of blocking uptake of noradrenaline by cocaine hydrochloride on the potentiating action of theophylline on the noradrenaline response. Cocaine in a concentration of 2.9×10^{-5}M was added to the perfusion fluid at the outset and all the dose response curves to

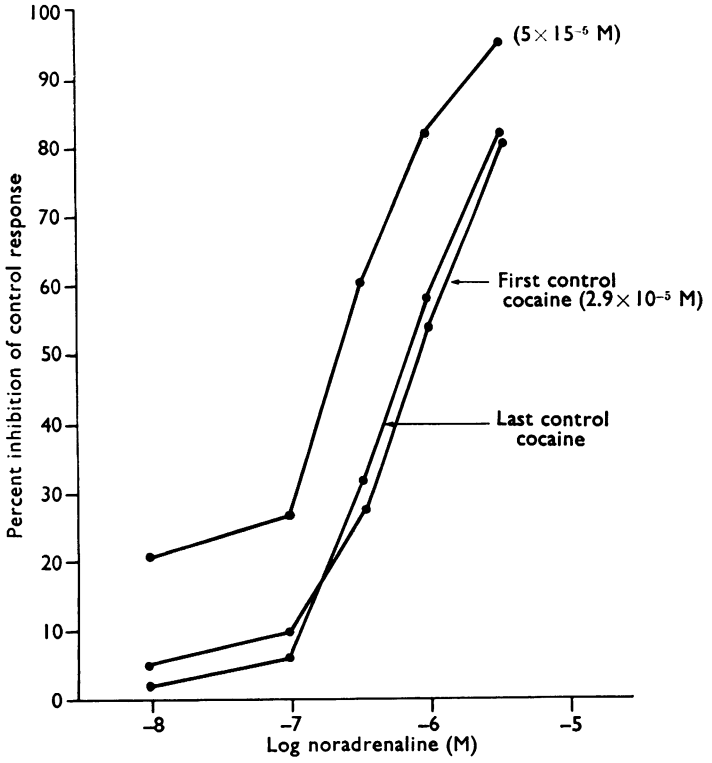

FIG. 2. Effect of theophylline (5×10^{-5}M) plus cocaine (10^{-5} g/ml.) treatment on the noradrenaline inhibition of the acetylcholine-induced contractions (1.1×10^{-5}M) in the isolated rat uterus. Log doses of noradrenaline were plotted against the per cent reduction in the acetylcholine-induced contraction. The first control dose response curve was determined before the addition of theophylline. The last dose response curve was determined after the removal of theophylline. Cocaine was present throughout the entire experiment. Each point represents the mean of eight experiments.

TABLE 3. *Effect of theophylline on the inhibitory response to nitroglycerine*

First control pD_2	Theophylline-treated (5×10^{-5}M) pD_2	Last control pD_2
3·83	4·25	3·90
3·83	4·08	3·95
3·94	4·26	4·13
4·19	4·45	4·18
3·69	3·88	3·60
3·66	3·90	3·73
3·58	3·83	3·85
3·83	4·13	3·78
Mean 3·813	4·09	3·89
±S.E. 0·068	0·074	0·069
P	*<0·05	>0·05

* Differs from control (paired *t* test).

noradrenaline before, during and after treatment with theophylline were determined in its presence. Following treatment with theophylline, as is shown in Table 2, the inhibitory responses to noradrenaline was potentiated approximately 3.4-fold. Figure 2 shows the shifting of the dose response curve for noradrenaline to the left during theophylline treatment and then back to the right after its removal by washing. These results indicate that theophylline produces essentially the same degree of potentiation of noradrenaline whether cocaine was present or not.

Effect of theophylline on the inhibitory response to nitroglycerine

In this series nitroglycerine was used in place of noradrenaline in the manner described above. Nitroglycerine produced an inhibitory effect similar to that of noradrenaline and was administered in a dose range of 10^{-6} to 3×10^{-4}M. Following theophylline treatment, the dose response curve for nitroglycerine was shifted to the left and the pD_2 value was significantly different from the first and last control which did not differ from each other. The pD_2 values are shown in Table 3. Figure 3 shows the effects of theophylline on the nitroglycerine dose response curve. Theophylline produced a 1.9-fold increase in the uterine inhibitory response to nitroglycerine. This is a significant increase in nitroglycerine considering that the last control dose response curve for nitroglycerine shifted back to the right and did not differ significantly from the first control.

Effect of nitroglycerine on the inhibitory response to noradrenaline

In this series nitroglycerine was substituted for theophylline. The effects of pretreatment with nitroglycerine in a concentration of 6×10^{-5}M on the dose response curves for noradrenaline were determined. This dose of nitroglycerine had no inhibitory effect on the contraction induced by acetylcholine. As is seen in Table 4, nitroglycerine, like theophylline, increased significantly the pD_2 value, indicating

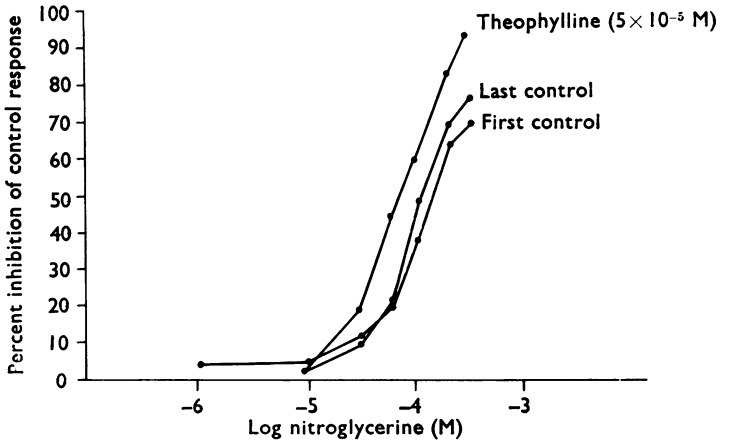

FIG. 3. Effect of theophylline (5×10^{-5}M) treatment on the nitroglycerine inhibition of the acetylcholine-induced contractions (1.1×10^{-5}M) in the isolated rat uterus. Log doses of nitroglycerine was plotted against the per cent reduction in the acetylcholine-induced contraction. The first control dose response curve was determined before the addition of theophylline. The last dose response curve was determined after the removal of theophylline. Each point represents the mean of eight experiments.

a potentiation of the noradrenaline inhibitory response. Figure 4 shows the shift of the dose response curve to the left after treatment with nitroglycerine, and after washing out the nitroglycerine the dose response curve for noradrenaline is then shifted back to the right. Nitroglycerine produced an approximate three-fold potentiation of the noradrenaline response.

TABLE 4. *Effect of nitroglycerine on the inhibitory response to noradrenaline*

First control pD_2	Nitroglycerine-treated (6×10^{-5}M) pD_2	Last control pD_2
5·80	6·05	5·78
4·55	6·25	5·53
5·90	6·15	5·88
5·80	6·05	5·88
5·70	5·98	5·80
5·75	5·78	5·55
5·68	5·98	5·60
6·05	6·23	5·75
Mean 5·59	6·06	5·72
±S.E. 0·17	0·05	0·05
P	*<0·05	>0·05

* Differs from control (paired *t* test).

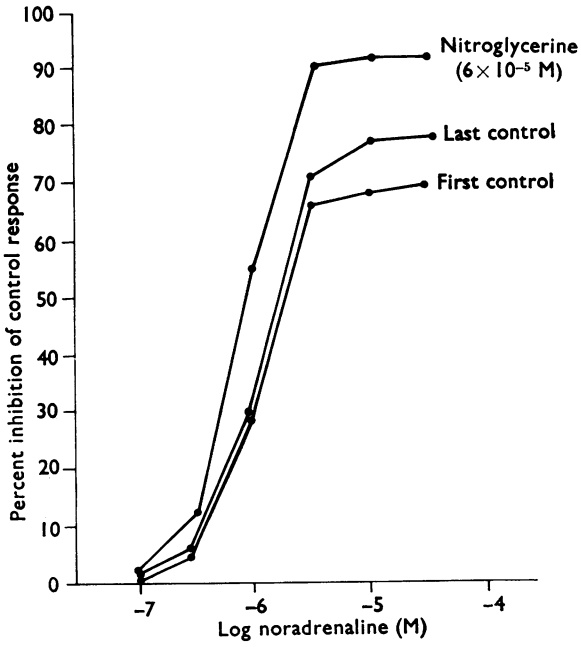

FIG. 4. Effect of nitroglycerine (6×10^{-5}M) treatment on the noradrenaline inhibition of the acetylcholine-induced contractions (1.1×10^{-5}M) in the isolated rat uterus. Log doses of noradrenaline were plotted against the per cent reduction in the acetylcholine-induced contraction. The first control dose response curve was determined before the addition of nitroglycerine. The last control dose response curve was determined after the removal of nitroglycerine. Each point represents the mean of eight experiments.

Discussion

Robison *et al.* (1967) have suggested that adenyl cyclase and the beta receptor are perhaps the same in all tissues, the intracellular mediator in this concept being cyclic AMP. Sutherland & Robison (1966) refer to cyclic AMP as the second messenger that mediates a variety of both metabolic and mechanical responses. The first messenger is the catecholamine that triggers this sequence of events by acting on adenyl cyclase. There is considerable evidence to support this hypothesis. The positive inotropic response to catecholamines has been studied most extensively in relation to this concept and there are many reports of the ability of catecholamines to produce myocardial contraction and an increase in cyclic AMP levels concomitantly and that both these effects are reduced by beta receptor blocking agents. In addition, the relative potencies of a series of catecholamines in stimulating adenyl cyclase were found to be similar to their relative potencies as inotropic agents *in vivo* (Murad *et al.*, 1962).

If this hypothesis is correct, then the level of cyclic AMP in intact tissues should increase in response to catecholamine stimulation. Moreover catecholamines which stimulate adenyl cyclase should be potentiated by drugs which inhibit phosphodiesterase activity. It is this last criterion that we are questioning by the results obtained in this study. Rall & West (1963) have reported that pretreatment with theophylline potentiates the inotropic response to norepinephrine in rabbit atria. Lundholm *et al.* (1966) have reported similar results in intestinal smooth muscle. Dobbs & Robison (1968), in a preliminary study, have suggested that the same relationship also exists in rat uterine muscle. Our results indicate that theophylline treatment does potentiate the response to noradrenaline and this would seem to confirm results reported by Dobbs & Robison (1968). The fact that theophylline was able to potentiate the rat uterine inhibitory response to nitroglycerine as well as to noradrenaline, however, would suggest the possibility of a non-specific potentiating action on the part of theophylline. This was further suggested by the observation that pretreatment with nitroglycerine also potentiated the noradrenaline response. Nitroglycerine has long been considered to exert non-specific smooth muscle depressant effects similar to those of theophylline. The studies performed with uptake mechanisms blocked by cocaine would rule out inhibition of noradrenaline uptake as a possible mechanism of noradrenaline potentiation by theophylline.

These results do not eliminate cyclic AMP as a mediator of catecholamine responses but they do raise serious doubts. Shanfeld *et al.* (1968) have reported that isopropylmethoxamine (Levy, 1964) abolished the elevation in phosphorylase activity and partially inhibited the rise in cyclic AMP but did not reduce the positive inotropic response to noradrenaline. This would suggest a possible separation of these responses. These results raise doubts of the validity of using theophylline as a potentiating agent particularly when theophylline itself produces the same effects as the agent it potentiates. It is interesting to speculate what effect theophylline would have on the positive inotropic effect to a non-adrenergic stimulus such as high K^+. Dean (1968) has reported that aminophylline in a dose range of 5–50 μg/ml. potentiated and markedly increased the inotropic and chronotropic responses to histamine in the isolated, perfused rabbit heart. In an earlier study, Trendelenburg (1960) concluded that histamine had a direct stimulant effect on cardiac tissues of cats, rabbits and guinea-pigs. This response was not abolished by antihistaminic

agents or by beta receptor blockade. In some preliminary studies, using anaesthetized dogs, we have been able to show a potentiation of the cardiac responses to sympathetic nerve stimulation with theophylline and in this preparation theophylline produced a positive inotropic effect of its own (our unpublished work). Diazoxide, an agent which, like theophylline, inhibits phosphodiesterase (Senft, 1968) but which has no significant positive inotropic effect, did not potentiate the positive inotropic or positive chronotropic responses either to sympathetic nerve stimulation or to injected noradrenaline.

These results do not support the hypothesis that beta receptor activation in the rat uterus by noradrenaline is specifically influenced by modifying cyclic AMP levels with theophylline.

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