Brit. J. Pharmacol. (1956), **11**, 99.

SLOW HYPERFIBRINOGENAEMIC ACTION OF ADRENALINE AND RELATED SUBSTANCES

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(RECEIVED JULY 1, 1955)

A single subcutaneous injection of adrenaline produces a rise in the concentration of plasma fibrinogen in the rat (Henriques, Henriques, and Mattos, 1950). This rise is proportional to the logarithm of the dose in the range 12.5–200 μg./100 g. body weight. A dose of 50 or 100 μg. adrenaline, given subcutaneously or intraperitoneally, caused a marked increase of fibrinogen 24 hr. later, but no change was detected 1, 2, or 4 hr. after administration. This lag between injection and response seems to distinguish the phenomenon—which had also been reported briefly by Henriques, Henriques, and Selye (1950)—from similar results recorded in the earlier literature. Thus, Riecker and Winters (1931), Goreczky and Kovats (1942), and Sütö-Nagy (1944) reported a transitory rise of plasma fibrinogen a few minutes after the administration of adrenaline to dogs or men. Biggs, MacFarlane, and Pilling (1947) have reported a transient increase in fibrinolysis after adrenaline administration to human subjects.

This paper describes experiments on the time-relation between treatment and appearance of fibrinogen increase, on the relation between chemical structure and hyperfibrinogenaemic action of sympathomimetic amines, and on the blocking by tolazoline ("Priscol") of the hyperfibrinogenaemic effect of adrenaline.

MATERIAL AND METHODS

The adrenaline used was either (±)-adrenaline hydrochloride (Sterling-Winthrop), or a 1:1,000 solution of "Adrenalin" (Parke, Davis & Co.). The latter was shown by chromatographic analysis to contain a small proportion of (−)-noradrenaline; Auerbach and Angell (1949) found from 10.5 to 18.5% of (−)-noradrenaline in different samples of U.S.P. adrenaline. The (±)-noradrenaline and *N-iso*propylnoradrenaline hydrochlorides used were also obtained from Sterling-Winthrop. Amphetamine hydrochloride was kindly supplied by Dr. M. Rocha e Silva. Other drugs used were histamine dihydrochloride (Paul-Lewis Laboratories); phenylephrine (Neosynephrine, Winthrop Products); pholedrine (Veritol, Knoll A.G.); ephedrine (British Drug Houses); and tolazoline (Priscol, Ciba). The hydrochlorides of tyramine, hydroxytyramine, *N*-methylhydroxytyramine, noradrenalone, *N*-butylnoradrenalone, *N*-methyladrenalone were synthesized in our laboratory by standard techniques. The purity of these substances was verified by melting point determinations and by chromatography on Whatman No. 1 filter paper, using butanol, acetic acid and water as solvent and $FeCl_3$-K_4[$Fe(CN)_6$] as developer (Barton, Evans, and Gardner, 1952). All substances gave only one spot on the chromatograms, except *N*-methylhydroxytyramine which contained traces of hydroxytyramine. Sometimes the purity was also checked by nitrogen determination. (−)-Adrenochrome was prepared by the method of Sobotka and Austin (1951); no adrenaline was detectable in it by the toad heart test.

Experimental Techniques.—Albino rats, 100–150 g. body weight, were used. To test the hyperfibrinogenaemic action, a substance was injected subcutaneously, the controls receiving an equal volume of 0.9% NaCl, and the animals were bled and killed on the next day. In the experiments done to determine the blocking activity of tolazoline on lung oedema produced by "Adrenalin," tolazoline was given subcutaneously 30 min. before the "Adrenalin" was injected intravenously through a tail vein. When testing the influence of tolazoline on the hyperfibrinogenaemic action of "Adrenalin," tolazoline was given 30 min. before "Adrenalin"; both drugs were given by subcutaneous injection. In a few experiments the treatment with tolazoline was repeated before the "Adrenalin" injection. The animals were always bled through the aorta, under pentobarbitone (40 mg./kg.) or ether anaesthesia, by means of a dry syringe coated with Dry Film 9987, according to the technique of Jaques, Fidlar, Feldsted, and MacDonald (1946). The blood was immediately transferred to a tube containing 0.01 ml. of 30% potassium oxalate/ml. blood and centrifuged. In a few experiments part of the blood was allowed to clot, the serum being used for the determination of total and non-protein nitrogen.

Analytical Methods.—For fibrinogen determinations, the fibrin, isolated from 1 ml. of blood plasma by the method of Cullen and Van Slyke (1920), was transferred to a Kjeldahl micro-flask and ashed by the method of Campbell and Hanna (1937). The ammonia thus formed was distilled and collected in excess standard acid, which was subsequently titrated. Finally ammonia nitrogen was converted into fibrinogen nitrogen using 6.00 as conversion factor (Morrison, 1947). By means of the same ashing and titration technique total nitrogen was determined in 0.1 ml. of blood serum, and non-protein nitrogen (NPN) was determined in 5 ml. of Folin-Wu blood serum filtrate (Folin and Wu, 1919). Protein nitrogen, obtained by difference between total nitrogen and NPN, was converted into the total serum protein by using 6.25 as conversion factor.

RESULTS

Influence of Time on the Hyperfibrinogenaemic Action of "Adrenalin."—Table I shows that "Adrenalin," 200 μg./100 g. body weight subcutaneously, caused a significant rise of fibrinogen as soon as 4 hr. after injection. After a single injection the fibrinogen concentration was still increasing between 8 and 12 hr. after treatment.

TABLE I

INFLUENCE OF TIME ON THE HYPERFIBRINOGENAEMIC ACTION PRODUCED IN THE RAT BY SUBCUTANEOUS ADMINISTRATION OF "ADRENALIN" (PARKE, DAVIS & CO.)

Dose (μg./100 g.)	Time After Injection (hr.)	No. of Animals	Plasma Fibrinogen (mg./100ml. ± S.E.)
200	4	9	318 ± 17·7
200	8	7	362 ± 9·5
200	12	9	507 ± 22·8
400*	24	6	699 ± 19·6
Saline control	24	10	255 ± 13·8

* Given in two doses, interval 3 hr., and the rats bled 24 hr. after the first injection.

Relation between Chemical Constitution and the Hyperfibrinogenaemic Action of Sympathomimetic Amines.—The following amines caused a significant rise in the concentration of blood plasma fibrinogen: Adrenaline was the most active; *N-iso*propylnoradrenaline, noradrenaline and phenylephrine were intermediate, and *N*-methylhydroxytyramine, adrenalone, hydroxytyramine and pholedrine were much less active (Table II). All the other compounds, including adrenochrome, proved to be inactive in the dose administered.

The relative potencies shown in Table II were calculated by dividing the dose of adrenaline, which should produce the same effect as the substance studied ("adrenaline equivalent"), by the dose of the sympathomimetic amine used in the experiment. The "adrenaline equivalent" is the antilog of $\left[\frac{F_U}{F_A}\log A\right]$, where F_U is the difference in blood fibrinogen concentration between the average of the groups treated with the amine of unknown activity and the average of the control group; F_A is the similar difference between adrenaline-treated and control groups; A is the adrenaline dose used in the corresponding experiment. Nevertheless this calculation can only give an approximation to the true relative potencies of the different substances. The design of the experiment would not allow an appraisal of the errors involved, and therefore differences as small as the one observed between (±)-noradrenaline and

TABLE II

HYPERFIBRINOGENAEMIC ACTION OF SYMPATHOMIMETIC AMINES

The potencies are related to that of (±)-adrenaline taken as 1 (see Results for method of calculation). All the rats were bled 24 hr. after a single subcutaneous injection of the amine.

Expt.	Substance	Dose (mg./100g.)	No. of Animals	Fibrinogen (mg./100 ml. ± S.E.)	*P* Related to Saline Control	Relative Potency
I	(±)-Noradrenaline	0·1	20	269 ± 10·7	0·002	0·04
	(±)-Adrenaline	0·1	21	361 ± 9·7	0·001	1
	(±)-*N-iso* propylnoradrenaline	0·1	20	335 ± 9·2	0·001	0·40
	Saline control	—	21	228 ± 6·6	—	
II	Phenylephrine	1	11	321 ± 14·9	0·001	0·03
	(±)-Adrenalinecontrol	0·1	12	353 ± 21·0	0·001	1
	Saline control	—	12	233 ± 7·2	—	
III	Hydroxytyramine	2	10	245 ± 13·7	0·007	0·001
	N-Methylhydroxytyramine	2	9	266 ± 16·1	0·001	0·002
	Tyramine	2	9	219 ± 6·9	0·048	0·001
	(±)-Adrenalinecontrol	0·2	9	507 ± 34·4	0·001	1
	Saline control	—	10	199 ± 6·8	—	
IV	Adrenalone	2	10	268 ± 10·0	0·008	0·002
	Noradrenalone	2	10	246 ± 12·3	0·19	0·001
	N-Butylnoradrenalone	2	9	223 ± 10·9	0·90	0
	N-Methyladrenalone	2	10	241 ± 10·6	0·24	0·001
	(±)-Adrenalinecontrol	0·2	10	430 ± 20·5	0·001	1
	Saline control	—	10	221 ± 12·3	—	
V	Amphetamine	1	10	223 ± 18·4	0·49	0·001
	Ephedrine	1	9	236 ± 17·2	0·14	0·002
	Pholedrine	1	10	253 ± 14·4	0·014	0·003
	(±)-Adrenalinecontrol	0·1	10	368 ± 17·8	0·001	1
	Saline control	—	10	209 ± 7·3	—	
VI	(−)-Adrenochrome	0·4	10	254 ± 14·0	0·23	0·005
	" "	0·8	10	254 ± 16·0	0·27	0·002
	(±)-Adrenalinecontrol	0·2	10	406 ± 12·5	0·001	1
	Saline control	—	9	230 ± 15·8	—	

TABLE III

INFLUENCE OF ADRENALINE ON THE CONCENTRATION OF PLASMA FIBRINOGEN, SERUM PROTEINS AND NON-PROTEIN NITROGEN

Adrenaline and saline were given in two subcutaneous injections, interval 5 hr., and the rats bled 24 hr. after the first injection. Number of rats used is indicated in parentheses

Expt. No.	(±)-Adrenaline (μg./100 g.)	Plasma Fibrinogen		Serum Proteins		Non-protein Nitrogen	
		mg./100 ml. ±S.E.	P	g./100 ml. ±S.E.	P	mg./100 ml. ±S.E.	P
I	180	484±13·7 (10)	0·001	6·06±0·01 (8)	0·27	31·9±1·27 (8)	0·007
	Saline control ..	196± 6·2 (10)		5·78±0·18 (9)		27·2±0·86 (9)	
II	200	488±29·2 (10)	0·001	6·24±0·23 (10)	0·10	—	—
	Saline control ..	189± 5·81 (10)		5·81±0·06 (10)			

phenylephrine (0.03 and 0.04) may not indicate a real difference in activity. The figures are therefore only rough estimates of the hyperfibrinogenaemic activity of the amines.

Influence of Adrenaline on the Plasma Fibrinogen, Serum Proteins and Non-protein Nitrogen (NPN) Concentration.—Adrenaline more than doubled the concentration of fibrinogen, producing at the same time a significant increase in the NPN concentration (Table III). In two experiments the total serum protein was higher in adrenaline-treated than in control animals, but this difference was not statistically significant.

Action of Other Nitrogenous Substances on the Concentration of Plasma Fibrinogen.—Tetraethylammonium and tolazoline did not affect plasma fibrinogen in a dose of 1 to 1.25 mg./100 g. body weight, whereas histamine increased it in a dose of 6.5 mg./100 g. but not when the dose was 0.65 mg./100 g. or less (Table IV).

Influence of Tolazoline on the Hyperfibrinogenaemic Activity of "Adrenalin."—In order to estimate the effective dose range of tolazoline in the rat, we measured its ability to prevent prompt death caused by the intravenous injection of "Adrenalin." Examination of the animals which died after intravenous injection of "Adrenalin" showed that they had lung oedema, since all of them presented a flow of foamy haemorrhagic fluid coming out of their noses. Preliminary experiments gave an LD50 for "Adrenalin" of 21 μg./100 g. (95% confidence limits 19.2–24.4 μg.). Table V shows the relation between the doses of tolazoline and the incidence of lung oedema in rats treated intravenously with 66 μg./100 g. of "Adrenalin." The mortality rate was 50% after treatment with 1.25 mg. tolazoline (95% confidence limits of 1.22 to 1.28). Therefore 1.25 mg. tolazoline seemed to have abolished the effect of 45 μg. "Adrenalin."

TABLE IV

PLASMA FIBRINOGEN CONCENTRATION OF RATS 24 HOURS AFTER THE SUBCUTANEOUS ADMINISTRATION OF SEVERAL NITROGENOUS SUBSTANCES

All the rats were bled 24 hr. after a single subcutaneous injection of the substance

Expt. No.	Substance	Dose (mg./150 g.)	No. of Animals	Fibrinogen (mg./100 ml. ±S.E.)
I	Tetraethylammonium	2	6	195± 3·17
	Tolazoline ..	2	6	202± 10·0
	"Adrenalin," P.D.	0·1	6	308±18·5
II	Histamine ..	0·02	11	182± 6·91
	,, ...	0·10	10	190± 7·76
	,, ...	1	11	211± 7·88
	,, ...	10	8	274± 7·11
	Saline control ..	—	8	218± 7·69

TABLE V

INHIBITION BY TOLAZOLINE OF THE LETHAL EFFECT OF INTRAVENOUS ADMINISTRATION OF 66 μG. "ADRENALIN" (PARKE, DAVIS) PER 100 G. OF BODY WEIGHT IN RATS

Tolazoline given s.c. 30 min. before "Adrenalin"

Dose of Tolazoline (mg./100 g.)	No. of Rats	Mortality %
0·83	10	80
1·00	19	63
1·17	10	50
1·42	10	60
1·67	10	30
2·00	10	10
4·00	10	0

The effect of pre-treatment with tolazoline on the hyperfibrinogenaemic action of "Adrenalin" was studied in several experiments. Judging from the ability of tolazoline to prevent death from "Adrenalin," the dose of tolazoline used should have been enough to inhibit the effect of the "Adrenalin" administered in all experiments except No. 1. The results (Table VI) show that in all experiments the average fibrinogen concentration of the tolazoline-"Adrenalin"-treated rats was smaller than that of the saline-"Adrenalin"-treated ones, although the difference was significant only in Expt. II and in one group of Expt. IV.

TABLE VI

INFLUENCE OF TOLAZOLINE ON THE SLOW HYPERFIBRINOGENAEMIC ACTION PRODUCED BY SUBCUTANEOUS ADMINISTRATION OF "ADRENALIN" IN THE RAT

The first tolazoline injection was given subcutaneously 30 min. before "Adrenalin" (Parke, Davis)

Expt. No.	No. of Rats	Dose (mg./150 g.) of		Fibrinogen (mg./100 ml. ±S.E.)
		Tolazoline (mg.)	"Adrenalin" (mg.)	
I	6	2	0·1	337±21·6
	7	—	0·1	382±27·9
II	10	3×2*	0·1	290±11·0
	10	3×2*	—	250±12·2
	10	—	0·1	347±12·0
III	7	4+2**	0·2***	315±15·0
	9	4+2**	—	272±17·0
	8	—	0·2	363±35·0
IV	8	4+2**	0·2	374±16·8
	7	4+2**	0·2	377±22·9
	8	6	0·2	376±14·9
	7	—	0·2	434±22·7
	8	4+2**	—	350±19·2

* Given in 3 doses, interval 2 hr. ** Given in 2 doses, interval 2 hr. *** "Adrenalin" was given mixed together with the first tolazoline injection. Sum of $\chi^2_8=22{\cdot}402$; $P=0{\cdot}01$ for comparison between saline-"Adrenalin"- and tolazoline-"Adrenalin"-treated rats. Sum of $\chi^2_6=19{\cdot}018$; $P=0{\cdot}002$ for the comparison between tolazoline-"Adrenalin"- and tolazoline-saline-treated rats.

On the other hand, the tolazoline-"Adrenalin"-treated rats had higher fibrinogen concentration than the tolazoline-saline-treated ones, but only in one experiment was the difference statistically significant. A χ^2 analysis of the results (Fisher, 1944) showed that tolazoline inhibits the hyperfibrinogenaemic action of "Adrenalin" ($\chi^2_8=22.402$; $P=0.01$), but this inhibition is not complete ($\chi^2_6=19.018$; $P=0.002$), with the dose administered in these experiments.

DISCUSSION

From our results, the hyperfibrinogenaemic action of sympathomimetic amines seems to be a consequence of very specific process. Thus, of the substances studied, adrenaline seemed to have the most suitable structure for hyperfibrinogenaemic action. Removal of one of its phenolic groups (phenylephrine) or of its *N*-methyl group (noradrenaline) decreased the potency to 1/20 to 1/30, and reduction of its alcoholic group to methylene (*N*-methyl-hydroxytyramine) or oxidation of the same group to ketone (adrenalone) reduced the activity to about 1/500. A substituent in the amine group improves the activity, for *N-iso*propylnoradrenaline, although less active than adrenaline, was more active than noradrenaline. This improvement in activity in the *N*-substituted derivatives of noradrenaline is a well-known feature of other pharmacological actions of adrenaline homologues, such as the inhibitory action on smooth muscle (Barger and Dale, 1910; Marsh, Pelletier, and Ross, 1948; Bacq and Lecomte, 1949; Bacq, Fischer, and Lecomte, 1949; Lands, 1949), on glycogenolysis (McChesney, McAuliff, and Blumberg, 1949; Ellis, 1951), and on the adrenaline-induced decrease in the ascorbic acid content of the adrenal glands (Jarrett, 1951). Of these, the activity which seems to be more closely related to the hyperfibrinogenaemic effect is the glycogenolytic action which can take place in the liver, where most of the fibrinogen is known to be formed (Miller, Bly, Watson, and Bale, 1951). McChesney *et al.* (1949) and Ellis (1951) observed that adrenaline possesses maximal hyperglycaemic activity, that noradrenaline has a potency of about 1/15 of this, and that *N-iso*propylnoradrenaline has very little activity. Therefore one could assume that, if both hyperfibrinogenaemic and hyperglycaemic activities take place in the same cells, the receptors involved should have different structural specificities; but for this assumption to be justified it would be necessary to show that the hyperfibrinogenaemic effect is caused by a direct action of adrenaline on the liver cells, as seems to be so far the hyperglycaemic effect (Cross and Holmes, 1937; Bendall and Lehmann, 1941). Tolazoline, however, seems to be able to block both the hyperfibrinogenaemic activity (present paper) and the hyperglycaemic activity of adrenaline (Ellis and Anderson, 1950; and Harvey, Wang, and Nickerson, 1952).

We found no change in the concentration of blood plasma fibrinogen in rats receiving 15 μg. "Adrenalin"/100 g. body weight, given into a tail vein. This result contrasts with that published by Henriques *et al.* (1950) showing an increase in fibrinogen after the subcutaneous administration of 12.5 μg./100 g. body weight, a discrepancy which might be explained by the very slow absorption of subcutaneous adrenaline (Cori and Cori, 1930a; Schayer, 1951). This, however, would not exclude the possibility of a direct effect on the liver cells, since the amine might work rather slowly; a better experiment would involve a prolonged intravenous infusion as done by Cori and Cori (1930b) to study the mechanism of adrenaline hyperglycaemia. Another possible explanation for adrenaline-induced hyperfibrinogenaemia would be the liberation of some active agent, set free by tissue damage caused by adrenaline ischaemia at the site of its administration, since it is well known that tissue damage causes an increase in the concentration of blood plasma fibrinogen (Foster and Whipple, 1922; Chanutin, Hortenstine, Cole, and Ludewig, 1938). Against this

interpretation we have the fact that in the rat, in the doses used by us, adrenaline does not produce any visible tissue damage. It is also noteworthy that *N-iso*propylnoradrenaline, which is considered a vasodilator agent (Konzett, 1940; Marsh *et al.*, 1948; Ahlquist, 1948), is the second most potent hyperfibrinogenaemic substance. In contrast, noradrenaline, which is generally admitted to have only vasoconstrictor activity, has a much lower fibrinogen-increasing effect. The conclusion as to the mechanism of adrenaline hyperfibrinogenaemia, whether by a direct action on the fibrinogen producing cells or by means of a mediator, must wait for more direct evidence.

The results presented suggest that the adrenaline-induced increase of fibrinogen is the consequence of a slow and very long process, since the concentration of fibrinogen was still rising after 12 hr. This might be explained by assuming that the normal metabolites of adrenaline are also active. Against this idea, adrenochrome, which has been considered an adrenaline metabolite (Bacq, 1949) and has some of the pharmacological actions of adrenaline (Bacq, 1947), did not produce any fibrinogen increase even in a dose of 800 μg. This, however, does not exclude the possibility of other metabolites being active, and indeed Schayer and Smiley (1953) have presented strong evidence that adrenochrome is not an intermediate in adrenaline metabolism.

In the experiments presented in this paper we have also found that the rats treated with adrenaline had a higher NPN concentration than the control animals. This confirms previous findings (Watkins and Smith, 1931).

Summary

1. A series of sympathomimetic amines and related substances were injected subcutaneously into rats. The following caused a significant rise in the concentration of blood plasma fibrinogen 24 hr. later: "Adrenalin," Parke, Davis & Co., (±)-adrenaline, (±)-*N*-*iso*propylnoradrenaline, (±)-noradrenaline, phenylephrine, *N*-methyl-hydroxytyramine, adrenalone, hydroxytyramine, and pholedrine. Adrenaline was the most potent, (±)-*N-iso*propylnoradrenaline had about 40%, and (±)-noradrenaline and phenylephrine had about 3%, of the activity of adrenaline.

2. Tolazoline (4 mg./100 g. body weight) partially abolished the hyperfibrinogenaemic effect of 100–200 μg. adrenaline.

Our thanks are due to Drs. M. Rocha e Silva and Ulla Hamberg for helpful criticism and to Dr. S. Schenberg for some pharmacological tests on the toad heart. The work was done during the tenure by one of us (A. L.) of a Brazilian Research Council Fellowship.

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