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EFFECT OF ADRENERGIC BLOCKERS AND RELATED COMPOUNDS ON THE TOXICITY OF EPINEPHRINE IN RATS

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Several investigators have demonstrated that adrenergic blocking agents antagonize the lethal effect of epinephrine on mice, (6, 9, 16, 18, 26) rats (20, 22) and other laboratory animals (1, 5, 11, 19, 22). In these studies, epinephrine and its antagonists were administered by various routes. The sensitivity of the methods used, which could be expressed by the reciprocal of the antidotal doses of standard adrenergic blocking agents, varied widely with the species and the route of administration of both epinephrine and the antagonists. The larger the dose of epinephrine required to kill a high percentage of animals, the larger were the antidotal doses. Due undoubtedly to a slow rate of absorption resulting from local vasoconstriction and biological decomposition, large doses of epinephrine were required to kill the animals when the intraperitoneal, subcutaneous or intramuscular routes of administration were used. The smallest LD_{50} values were obtained, as expected, when administration was made by the intravenous route but the magnitude of the doses varied considerably depending on the species used. Of four species of small laboratory animals (mice, rats, guinea pigs and rabbits), mice are the least and rats the most sensitive to the lethal effect of epinephrine. (A rat/mouse intravenous toxicity ratio of 60 was found by HOPPE, SEPPELIN and LANDS, 13). The low sensitivity of mice towards epinephrine and the use of routes other than the intravenous may account for the fact that in one investigation piperoxan was found to be ineffective as an antidote against epinephrine, even in doses as high as 50 mg/kg (14). The aforementioned considerations suggested the desirability of using the rat as the test animal and the intravenous

route in trying to measure the antagonistic activity of various compounds against the lethal effect of epinephrine.

Epinephrine and the antagonist were injected in the same solution. This was based on the assumption that a) both the agonist and the antagonist act rapidly, b) the maximal blood concentration occurs shortly after injection, decreasing considerably within a few minutes (the decrement is probably less rapid with most of the antagonists) and thus providing the maximal blood concentration of the antagonist during the time at which epinephrine reaches its highest concentration; and c) if the two drugs were not injected simultaneously, not only would it have been very difficult to maintain the same interval between the drugs administered in all the tests but differences in the blood concentration time curves of the antagonists would have been reflected in the estimates of relative activity. The results appear to justify the aforementioned assumptions regarding the sensitivity of the method, some of the compounds tested being effective in doses as low as 10 microgm/kg.

METHOD

The test animals were unfasted male albino rats of the Sprague-Dawley strain with a weight range of 80-120 gms. The rat was held in a metal cylindrical holder. Injections were made into one of the tail veins after warming the tail by immersion for 5-10 seconds in water at approximately 50° C. The volume injected was always 0.2 ml/100 gm of body weight injected in 2 or 3 seconds, using a 0.25 ml tuberculin syringe and a sharp 27 gauge one-half inch long needle.

Epinephrine (Suprarenin bitartrate) and the test drug were injected in the same solution. The dose of epinephrine (in terms of the base) was 200 micrograms (mcg.) per kg. This dose was 2.7 times larger than the intravenous LD_{50} of epinephrine base which was determined by the injection of 4 doses graded at 0.2 log intervals (TABLE I). The percent mortality, changed to probits, was plotted against the log of the concentration. The standard error was estimated graphically by a slightly modified MILLER and TAINTER method, the modification consisting in using for all the calculations the log of the doses in the line of abscissae instead of the antilogs. The standard error (s.e.) obtained in this manner differed only slightly from that estimated by MILLER and TAINTER's method for s.e. below 15 % of the mean.

The stock solution of epinephrine was made in a 1 : 10,000 dilution of ascorbic acid in distilled water. The test drug was also dissolved in

1 : 10,000 ascorbic acid. The pH of the solution was 5.5-6.5. One-tenth N HCl or 0.1 N NaOH was added when necessary. All the dilutions were made with the ascorbic acid solution. The final solution was prepared by mixing epinephrine and the test drug solution in such a proportion that each ml of the mixture contained 100 mcg of epinephrine base (one-half of the dose/kg) and one-half of the per kg dose of the drug (one-fifth of a ml was injected for one-tenth of a kg of body weight).

After the injection the rats were placed together with other injected rats. The control rats injected with 200 micrograms of epinephrine (base)/kg and most of the unprotected rats injected with the mixture died within a few minutes. Death was apparently due to pulmonary edema. (In the rats which were autopsied, a reddish frothy material flowed from the incised lungs). In only a small percentage of the rats was death delayed up to several hours. Pilo-erection, exophthalmus, blanching of the skin, was observed after the injection of epinephrine or the mixture in most of the rats examined, including the survivors. Three to five doses of the test drug graded at 0.3 log intervals, were administered. Ten or more rats (usually 15 or 20) were injected with each dose. The percent mortality (up to 24 hours) transformed to probits was plotted against the dose on probit log paper. The Effective $Dose_{50}$ (ED_{50}) was calculated from the linear regression line. The *s.e.* was estimated as described above.

Reserpine, phenoxybenzamine and Win 13,645 were injected also at various time intervals before epinephrine.

No attempt was made to obtain an ED_{50} value with methacholine. The following drugs were used :

Chlorpromazine HCl (Thorazine HCl, Smith Kline and French Labs.).
Dihydroergocornine methane sulfonate (Sandoz Chem. Works, Inc.).
Ergonovine maleate (Ergotrate maleate, Lilly).
Ergotoxine ethanesulfonate (Burroughs Wellcome).
D-Lysergic acid diethylamide.
2-Brom-D-lysergic acid diethylamide (Sandoz Pharmaceuticals, Hanover, New Jersey).
Piperoxan HCl (Piperidylmethyl-benzodioxane, Merck and Co.).
Phentolamine (Regitine, Ciba Pharmaceutical Prod., Inc., New Jersey).
Promazine HCl (Wyeth Inst. for Med. Research).
Tolazoline HCl (Priscoline HCl, Ciba).
Yohimbine HCl (The New York Quinine and Chemical Works, Inc.).
Reserpine (Penick and Co.).

Phenoxybenzamine HCl (Dibenzyline, Smith, Kline and French Labs.).
Phenindamine (Thephorin, Hoffmann-LaRoche Inc., Nutley, N. J.).
Methacholine chloride (Mecholyl chloride, Merck and Co., Rahway, N. J.).
Win 13,645. 8-{-3[10-(2-chlorophenothiazinyl)]propyl}-3-hydroxynortropane.
Win 14,020. 8-{-3[10(2-chlorophenothiazinyl]propyl-pseudo}-3-hydroxynortropane.
(The bases or the ethanesulfonate salts of Win 13,645 or Win 14,020 were used).

Results and Comments

With the exception of reserpine, all the compounds in this series antagonized the lethal effect of 200 mcg/kg of epinephrine. The ED_{50} represents the dose of the compound estimated from the dose-effect curve that reduced the toxicity of the aforementioned dose of epinephrine to that of one LD_{50}. The toxicity of epinephrine was first determined at the beginning of our studies in the month of July. In order to investigate possible seasonal variations of sensitivity to epinephrine, similar tests were carried out in December and March. The results are presented in Table I. No significant differences were found between the ED_{50}

Table I

Intravenous Toxicity of Epinephrine in Rats Tests Run at Various Times of Year

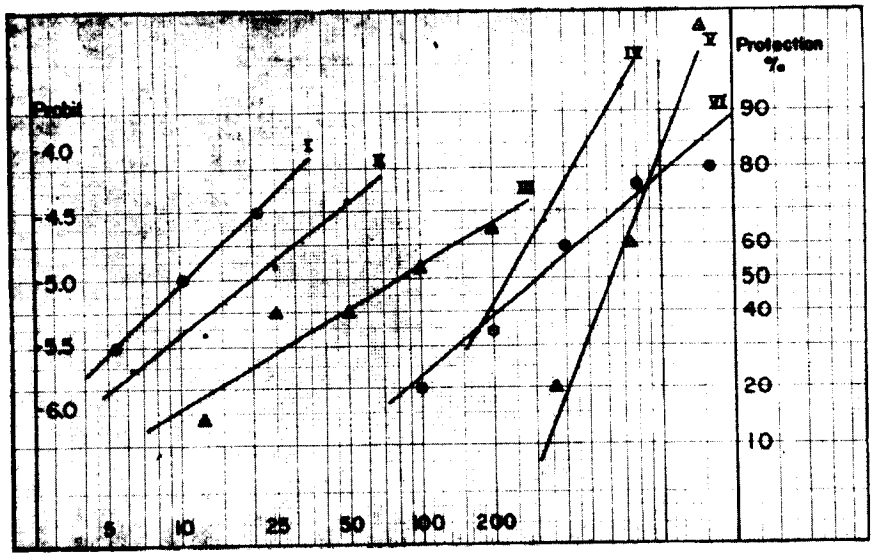
Month	Number of Doses (1)	Number of Rats/Dose	Toxicity in mcg/kg	
			ED_{50}	ED_{50} + and — S.E.
July	4	15	73	79.6 — 66.9
December	3	15	74	80.4 — 68.1
March	4	10	78	90.1 — 67.6
July-Dec.,-Mar.	4	40, 40, 40 and 25	75	79.5 — 70.8

(1) Doses graded at 0.2 log intervals.

values obtained at various times. The value of 75 mcg/kg was obtained from a dose-effect curve which combined the results of the three separate tests. This value differed significantly from the LD_{50} reported by HOPPE *et al.* (40 $\pm$ 4 micrograms/kg) obtained with Webster strain rats (13) (1). The results are shown in Table II.

Relative antidotal activity has been calculated in terms of percentage of the activity of chlorpromazine, activity being expressed as the reciprocal of the LD_{50}.

Antagonism of the lethal effect of epinephrine in rats
(Dose of Epinephrine = $LD_{50} \times 2.7$)



Dose of antagonists : micrograms Kg.

FIG. 1

Percentage of rats protected with graded doses of epinephrine antagonists. Epinephrine (200 micrograms/kg) was administered intravenously in the same solution with the antagonist.

I = Dihydroergocornine, II = Win 14,020, III = Phenindamine, IV = Piperoxan, V = Yohimbine and VI = LSD.

The most active drugs were dihydroergocornine, phentolamine, and the phenothiazine compounds (chlorpromazine, Win 14,020 and Win 13,645). The last two compounds whose adrenergic blocking activity in dogs have been reported by LONG, LANDS and ZENITZ (15), are isomers : apparently anti-epinephrine activity in this case is not affected by the steric differences between tropane and pseudo-tropane. Our list

(1) See addendum p. 120.

includes five of the compounds that were tested by FLECKENSTEIN (8) for antiepinephrine effect on the vessels of the rabbit ear. With the

TABLE II

Antagonism of Epinephrine Toxicity in Rats
(Antagonist injected simultaneously with 2.7 × LD_{50} of epinephrine)

Compound	No. of Doses	Total No. of Rats	ED_{50} mcg/kg (1)	ED_{50} ± S.E. mcg/kg (1)	Approx. Relative Activity Chlorpromazine = 100
Chlorpromazine HCl	4	100	21.	26.1–16.9	100.
Dihydroergocornine (Methane sulfonate)	3	30	11.9	16.5–8.6	180.
Ergonovine maleate	4	60	85.	103–70.4	25.
Ergotoxine ethane sulfonate	4	40	51.	63–41.	41.
D-Lysergic acid diethylamide (LSD)	4	50	320.	417–245	6.6
2-Brom-D-Lysergic acid diethylamide (Br.LSD)	4	60	245.	300–200	8.6
Piperoxan HCl	3	45	240.	280–206	8.7
Phentolamine HCl	3	45	14.8	17.9–12.1	140.
Phenindamine tartrate	5	75	80.	112–57	26.
Phenoxybenzamine	4	20	31.	41–24	67.
Promazine HCl	3	30	41.	52–30	51.
Tolazoline HCl	4	80	600.	708–509	3.5
Yohimbine HCl	3	45	600.	646–565	3.5
Reserpine	3	10	Inactive at 1000		
Win 13,645	3	105	16.7	20–14	125.
Win 14,020	4	155	20.2	23–17.7	100.

(1) Doses in terms of the bases.

exception of piperoxan we have ranked them in the same order of activity : Phentolamine >phenindamine >yohimbine >tolazoline.

The anti-epinephrine activity of D-lysergic acid diethylamide (LSD) was much higher than expected in view of its potentiation of the pressor effect of epinephrine as reported by VALDECASAS and his co-workers (27) and the low anti-epinephrine activity found by GINZEL and KOTTEGODA (10) and by ROTHLIN (23). 2-Brom-LDS was found slightly more active than LSD, but the difference was not significant. This was much lower than the 10 : 1 ratio found by ROTHLIN (30) on the guinea pig seminal vesicle. Phenoxybenzamine showed an unexpectedly high activity when injected simultaneously with epinephrine, although its potency increased approximately 3 times when injected 40-80 minutes before the agonist (TABLE III). This may indicate a much faster formation of the ethylenimonium derivative after intravenous injection in the rat than in the *in vitro* experiments of HARVEY and NICKERSON (12). On the other hand, in the case of Win 13,645 previous administration (15 to 70 minutes)

TABLE III

Antagonism of Epinephrine Toxicity in Rats

Compound	Time Interval Between Drugs (min) (1)	No. of Doses	Total No. of Rats	ED_{50} mcg/kg	ED_{50} ± S.E. mcg/kg
Phenoxybenzamine (N-Phenoxy-iso propyl N-benzyl-beta-chlorethyl-amine) HCl	0	4	20	31.	(41–24)
	40–80	3	34	8.9	(10.3–7.7)
Win 13,645	0	3	105	25.	
,,	15–35	4	38	47.	(58.5–37.7)
,,	45–70	4	35	91.	(12.2–56.6)
Reserpine	0	1	10	Inactive at 1000	
,,	30	1	3	,, ,, 400	
,,	120	1	3	,, ,, 1000	
,,	180	1	5	,, ,, 1000	

(1) Antagonists were injected simultaneously with (time interval = 0) or before 2.7 · LD_{50} epinephrine.

is less effective than simultaneous injection with epinephrine (TABLE III). Doses of epinephrine larger than 2.7 times the LD_{50} could be antagonized by Win 13,645 or Win 14,020 in doses proportionally larger than their respective ED_{50}. Even a dose equivalent to a 99 LD_{50} of epinephrine failed to kill the majority of rats when administered in the same solution with a dose of 2,400 mcg/kg of Win 13,645 ($ED_{50} \times 144$) or 2,150 mcg/kg of Win 14,020 ($ED_{50} \times 106$).

Methacholine, in doses of 250, 1000 and 2000 mcg/kg tested with epinephrine as described above (5 rats/dose) protected 4, 5 and 4 rats respectively. This confirms results obtained by LEOW and MICETICH (14) in mice.

Although no attempt was made to investigate the mechanism of the anti-epinephrine effect studied, our results and those reported by other investigators suggest that, with the exception of methacholine, protection was the result of peripheral antagonism of the vasoconstrictor action of the compounds tested.

Intense and sustained high arterial blood pressure with increased left ventricular work load leading to an elevated pulmonary capillary pressure

TABLE IV

Antagonism of Epinephrine Toxicity in Rats

(Antagonist injected simultaneously with 9.9 to 99 $\times$ LD_{50} of Epinephrine)

Compound	Dose of Antagonist (1)		Dose of Epinephrine		Mortality	
	mcg/kg	Number of ED_{50}	mcg/kg	Number of LD_{50}	No. Died/ No. Tested	Per Cent
Win 13,645	240	14.4	740	9.9	3/10	30
	192	11.5	800	10.7	0/5	0
	192	11.5	1,600	21.3	0/5	0
	192	11.5	3,200	43.	2/5	40
	480	28.7	3,700	49.	5/5	100
	2,400	144.	7,400	99.	5/15	33
Win 14,020	215	6.	740	9.9	3/10	30
	172	8.5	800	10.7	0/5	0
	172	8.5	1,600	21.3	0/5	0
	172	8.5	3,200	43.	2/5	40
	430	21.2	3,700	49.	4/5	80
	2,150	106.	7,400	99.	4/15	27

(1) Epinephrine and the antagonist were injected in the same solution.

are adequate to explain the occurrence of lung edema shortly after the intravenous injection of epinephrine, as reported by VISSCHER, HADDY and STEPHENS (28) and CHENG (4). In the rabbit, DRENCKHAHN (7) has shown that following the intravenous injection of large doses of epinephrine the pulmonary venous pressure (p.v.p.) increases in most cases when the mean arterial pressure exceeds 130 mm Hg and that lung edema (measured by lung weight) occurs when the p.v.p. is higher than 30 mm Hg. In those cases the lung weight was roughly proportional to the degree of the rise of the p.v.p. above the threshold value of 30 mm Hg.

Although a central site of action for epinephrine and the adrenergic blocking agents has been suggested (CASSEN *et al.*, 2, 3) the experiments of PAINE *et al.* (20) showing that clamping of the aorta in the dog could produce lung edema, and other studies reviewed by VISSCHER *et al.* (28) strongly suggest that only the peripheral action of epinephrine is involved.

The adrenergic blocking agents would act as antidotes by partial block at the epinephrine receptor site and thereby reducing the magnitude and/or the duration of the arterial pressure rise. Physiological antagonism of the action of epinephrine on the cardiovascular system suffices to explain the action of methacholine (LEOW and MICETICH, 16). Phenindamine, a potent antihistamine (LEHMAN, 14) showed a high degree of anti-epinephrine activity. The latter effect should be attributed to its adrenergic blocking activity, demonstrated on the isolated ear vessels by FLECKENSTEIN, who also found that there was no correlation between the antihistamine and the anti-epinephrine activity of phenindamine, tolazoline and phenergan. However, another possibility must be considered. HALPERN *et al.* (11) found that phenergan inhibits the lung edema which follows the inhalation of chloropicrine as well as that produced by the injection of epinephrine. They hypothesized that the antidotal effect was due to a direct effect of phenergan on lung capillary permeability. On the other hand phenergan is known to have adrenergic blocking activity and this alone could explain its action in epinephrine induced edema but the possibility of an additional protective action by a direct effect on capillary permeability can not be excluded.

The results obtained with methacholine demonstrated that the method described is not specific for adrenergic blocking activity. In addition to the protective action against the lethal effect of epinephrine, adrenergic blocking activity should be demonstrated by the usual tests on the blood pressure of dogs or cats. With those limitations, the antidotal effect on rats could be used to quantify adrenergic blocking activity.

Another objection that can be raised against the use of mixed solutions is the possibility of chemical or physico chemical incompatibility. With the compounds studied, we did not observe discoloration or any other physical change after mixing the agonist and the antagonist solutions. We tested on the blood pressure of dogs mixtures of epinephrine with the following antagonists: ergonovine, chlorpromazine and phenoxybenzamine in the proportions which afforded nearly a complete protection in the rat. (These solutions, which also contained ascorbic acid 1 : 10,000, were kept at room temperature for 10 or more minutes before injection). Because the dose of antagonist was too small when epinephrine was injected at 1, 2 or 4 mcg/kg, we could compare the pressor effect of epinephrine with control doses and demonstrate that no inactivation of epinephrine had occurred.

Summary

1. Sixteen compounds were tested as antagonists of the lethal effect of epinephrine in rats.

2. Epinephrine and graded doses of the experimental drug were administered in the same solution by intravenous injection. The dose of epinephrine was 200 mcg/kg or 2.7 times larger than the LD_{50}.

3. The ED_{50} was calculated from the dose-mortality (per cent) curve.

4. The following ED_{50} values (in mcg of base/kg) were obtained: Chlorpromazine 21, dihydroergocornine 10.7, ergonovine 85, ergotoxine 51, L.S.D. 320, 2-Bromo-L.S.D. 245, piperoxan 240, phentolamine 14.8, phenindamine 80, phenoxybenzamine 31, promazine 41, tolazoline 600, yohimbine 600, Win 13,645 16.7, and Win 14,020 20.2. Reserpine was inactive at a dose of 1,000 mcg/kg whether injected in the same solution with or 3 hours before epinephrine.

Addendum

Recently (Feb. 1959) an LD_{50} value of 45.5 ± 2.5 mcg/kg (in terms of the base) was obtained with the same sample of epinephrine bitartrate, using the same technique, apparently the same strain of rats and 40 animals per dose. Obviously, the toxicity of epinephrine should be determined at intervals while measuring epinephrine antagonism of the type described in this paper. In addition, a well-known adrenergic blocking agent should be tested.

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