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THE INCREASE IN THE TOXICITY OF YOHIMBINE INDUCED BY IMIPRAMINE AND OTHER DRUGS IN MICE

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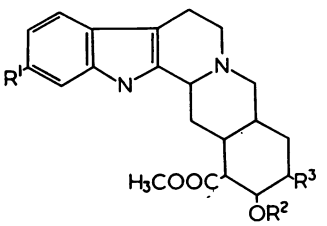
In mice, yohimbine appears to accentuate the normal "alarm" reactions (alerting, flight) to external stimuli. Imipramine increases this effect and at the same time converts a non-lethal dose of yohimbine into a lethal one. The effect of imipramine is greatly reduced by adrenalectomy or by treatment with reserpine, syrosingopine, ganglion-blocking drugs or adrenaline antagonists acting on sympathetic β-receptors. Hypnotic, anti-convulsant or anaesthetic agents, tetrabenazine or antagonists of 5-hydroxytryptamine do not reduce the imipramine effect. A variety of drugs which, like imipramine, are known to interfere with the tissue binding of noradrenaline also increase the toxicity of yohimbine. Yohimbine significantly reduces brain noradrenaline content; adrenal catechol amines are slightly reduced. The results suggest that yohimbine releases noradrenaline from stores or nerves as a consequence of increased central sympathetic activity. Imipramine increases the actions and toxicity of yohimbine by increasing the effects of the released noradrenaline on β-receptors. The lethal effects of a high dose of yohimbine alone are not reduced by any of the treatments tested, and appear not to result from activation of sympathetic mechanisms.

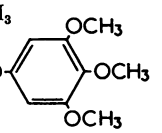
Symptoms of central autonomic and psychic stimulation after administration of yohimbine to unanaesthetized animals and man have often been reported but have been little studied. The alkaloid's aphrodisiac action is considered to depend partly on a peripheral vasodilatation in genital organs and partly on a stimulation of higher nervous centres affecting sexual activity, since penile erection is abolished by light anaesthesia (Müller, 1907). Various workers have reported hypertension, hyperventilation, salivation, diarrhoea and symptoms of uneasiness, anxiety and increased alertness and reflex excitability in animals. Higher doses of yohimbine produce muscle tremors, ataxia, excitement and finally convulsions (Oberwarth, 1898; Müller, 1907; Weger, 1931; Weinberg, 1933; Gershon & Lang, 1962); these effects are not seen in anaesthetized preparations, in which injection of the alkaloid produces a fall, instead of a rise, of blood pressure.

Recently attention has been drawn to the possible use of the drug in man as a tool for assessing individual emotional reactivity, since this appeared to influence the degree of the autonomic and emotional changes seen in patients given a test dose of the alkaloid (Holmberg & Gershon, 1961; Holmberg, Gershon & Beck, 1962). In the course of their investigations, these authors observed that both the autonomic and in particular the psychic effects of yohimbine were potentiated in patients receiving treatment with the anti-depressant drug imipramine.

The interaction between these two drugs has been studied in the hope that some light might be thrown upon the mechanisms of action of both yohimbine and imipramine, and that the results thus obtained might form the basis of a specific screening test for anti-depressant compounds possessing imipramine-like activity. Other drugs used clinically in the treatment of depressive states, such as amitriptyline, amphetamine and inhibitors of monoamine oxidase, have also been examined, as well as certain phenothiazines and other agents capable of modifying autonomic nervous activity. In addition, a degree of similarity in pharmacological action



Yohimbine
$R^1 = H$
$R^2 = H$
$R^3 = H$

Reserpine
$R^1 = OCH_3$
$R^2 = CH_3$
$R^3 =$ 

has been observed between yohimbine and reserpine which may be related to a certain similarity in their chemical structure. Traces of yohimbine are found in many species of *Rauwolfia,* including *R. serpentina* (for references, see Bein, 1956).

METHODS

Toxicity studies. Albino male mice (T.T. strain), 18 to 25 g of body weight, were used. Mice were injected with yohimbine in the afternoon and placed five in a compartment about 12 cm square; the mortality rate was assessed the following morning. Room temperature was maintained at 23±2° C. Where the effects of treatments with various drugs were compared, comparisons were based on mortality figures for not less than ten mice in each test and control group, tested on at least two different occasions, and the significance of any difference assessed by a χ^2 test. Unless otherwise stated a subcutaneous dose of 20 mg/kg of yohimbine hydrochloride was used. This dose of yohimbine by itself was not lethal (see Results).

Imipramine and related compounds were administered orally 1 hr before the yohimbine; other drugs were given as indicated in the text. In experiments where adrenalectomized mice were used, the operation was performed on mice anaesthetized with ether or pentobarbitone (50 mg/kg) 24 hr before the test.

Note with regard to toxicity studies. In experiments concerning toxic interactions of two or more drugs, care was taken to check that the doses of each drug were not lethal by themselves. This did not of course rule out the possibility that an observed increase in the toxicity of yohimbine might be due simply to a summation of non-specific sub-lethal effects, and this might well have occurred in tests with adrenaline antagonists which increased the toxicity significantly only in fairly high doses. In most other experiments, however, drugs acted at relatively low doses, and a synergism was indicated.

Heart-rate. The heart-rate was measured in albino rats (male, 200 to 250 g) anaesthetized by an intraperitoneal injection of allobarbitone (32 mg/kg) and urethane (740 mg/kg). The anaesthetized animals were placed on a table warmed to 31 to 32° C. Electrodes made from no. 16 gauge hypodermic needles were inserted under the skin and the heart-rate was measured

from records of the electrocardiogram on a pen-writing oscillograph. The heart-rates of thirty-six rats were recorded in each experiment; responses to treatment with each drug represent the means of values from six to fifteen animals.

Determination of tissue amine contents. Mice (17 to 25 g) were killed by dropping them into a solid carbon dioxide-acetone freezing mixture; the adrenal glands and brain were removed and homogenized in 0.01 N- and 0.1 N-hydrochloric acid respectively. Catechol amines were estimated fluorimetrically by the semi-automatic method of Merrills (1962), and 5-hydroxytryptamine by the method of Kuntzman, Shore, Bogdanski & Brodie (1961). For each determination of adrenal or brain amine content, both adrenal glands from each mouse or brains from three or four animals were used.

Recoveries of noradrenaline added to brain homogenates and of adrenaline added to adrenal homogenates did not fall significantly below 100% (thirteen estimations); recoveries of 5-hydroxytryptamine added to brain homogenates averaged 75.5% (standard error, ±2.5%) and values for tissue amine content have been corrected accordingly. In the determination of brain noradrenaline, the use of thioglycollic acid as a stabilizing agent made this method specific for the estimation of noradrenaline.

In the 5-hydroxytryptamine determinations, since no spectrophotofluorimeter was available, an interference filter with a half-band width of 18 mμ was used to isolate the emitted light at 550 mμ. The possible presence of interfering substances was investigated by adding small amounts of each drug tested to the homogenized brains of untreated mice. In the case of yohimbine and tetrabenazine, interfering fluorescence was observed, and extracts of brains taken from mice treated with these two drugs gave values of up to 300% above normal (see Results).

Inhibition of monoamine oxidase activity. The brains of groups of three mice (30 to 40 g) were homogenized together in 0.1 M-phosphate buffer and the monoamine oxidase activity determined colorimetrically by the method of Green & Haughton (1961). Twelve mice received yohimbine hydrochloride (60 mg/kg) subcutaneously 1 to 2 hr before death; another twelve mice received saline. Those animals which had not already died from the effects of the yohimbine were anaesthetized with ether and decapitated. The cerebral hemispheres were removed from the brains before homogenization since they contain relatively little monoamine oxidase (Udenfriend, Weissbach & Bogdanski, 1957).

RESULTS

Behavioural and autonomic effects of yohimbine in mice

A dose of 20 mg/kg of yohimbine produced ptosis and slight sedation; the effects lasted for 2 to 4 hr. This dose was not lethal by itself (none of fifty-one treated mice died). Higher doses of yohimbine caused prostration and somewhat heightened sensitivity to external stimuli such as movement of another mouse. About 30 min after injection, mice began to exhibit intermittent bouts of clonic convulsions which were sometimes precipitated by external stimuli. These convulsions often continued intermittently for several hours; there was no final tonic phase even when the convulsions proved fatal.

The effects of yohimbine differed from those of an amphetamine-like central nervous stimulant by the absence of any continuous hyperactivity. Grouping of the mice did not significantly increase the mortality caused by yohimbine alone, at either the LD50 or the LD80 level ($P>0.30$).

Toxicity data obtained for yohimbine under the standard experimental conditions of grouping, time of injection, etc., are given in Table 1. The LD50 value calculated from the weighted regression line is 43.9 mg/kg.

TABLE 1
THE TOXICITY OF YOHIMBINE HYDROCHLORIDE IN MICE
Doses were injected subcutaneously

Dose (mg/kg)	No. of mice	
	Injected	Killed (%)
20	51	0
30	25	16
40	35	49
60	45	76

Increase in the toxicity of yohimbine caused by imipramine and related drugs

Treatment of mice with imipramine (2 to 50 mg/kg) orally 1 hr before a dose of yohimbine (20 mg/kg), which was not lethal by itself, produced toxic effects. After a dose of imipramine of 10 mg/kg, for instance, the consequent mortality due to 20 mg/kg of yohimbine was as great as that produced by about 50 mg/kg of yohimbine alone. The mortality values for various doses of imipramine when given before yohimbine (20 mg/kg) are represented in Fig. 1 ; the log dose-probit transformation satisfies the usual tests for linearity of regression. As after high doses of yohimbine alone, death was immediately preceded by clonic convulsions, but in the animals previously treated with imipramine the convulsive phase occurred as a climax to a bout of sudden twitching and jerking movements which were often precipitated by disturbance by another mouse, and was often rapidly fatal. Before

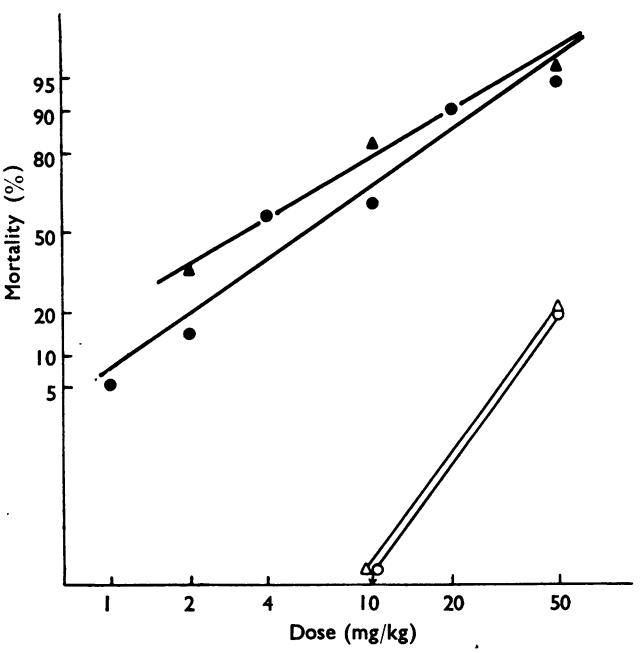


Fig. 1. The increases in the toxicity of yohimbine hydrochloride (20 mg/kg subcutaneously) in mice caused by imipramine (●) and by amitriptyline (▲). Open symbols indicate responses in mice previously treated with reserpine (5 mg/kg intraperitoneally).

this convulsive phase, which usually did not occur until 1 to 2 hr after injection of yohimbine, the mice appeared somewhat apprehensive and displayed salivation, increased depth of respiration and piloerection. The toxicity of imipramine (20 mg/kg) plus yohimbine (20 mg/kg) was significantly reduced by keeping the mice singly after injection ($P<0.02$).

Desmethylimipramine, in which form much of injected imipramine is reported to be present in the brains of treated animals (Gillette, Dingell, Sulzer, Kuntzman & Brodie, 1961), was as active as the parent compound in increasing the toxicity of yohimbine.

Amitriptyline, chlorpromazine and chlorprothixene increased the toxicity of yohimbine similarly to imipramine (Figs. 1 and 2). The ED50 values (that is doses

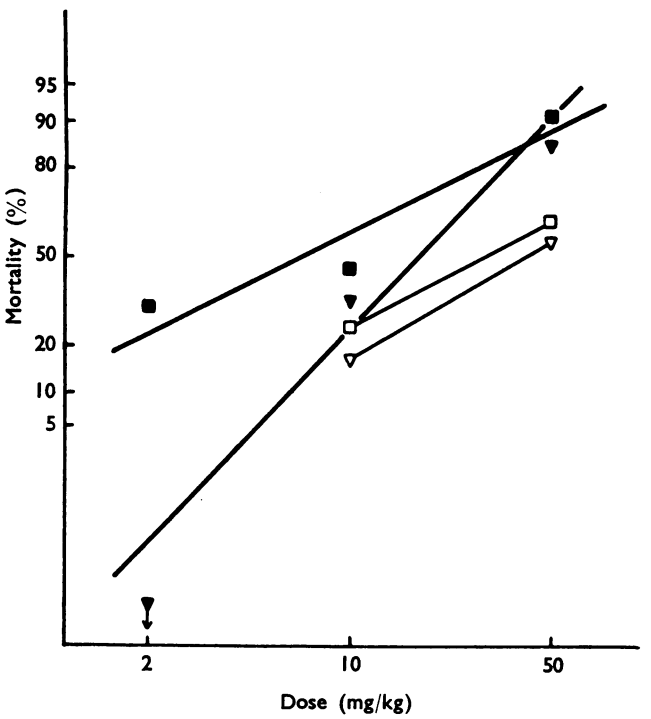


Fig. 2. The increases in the toxicity of yohimbine hydrochloride (20 mg/kg subcutaneously) in mice caused by chlorpromazine (■) and by chlorprothixene (▼). Open symbols indicate responses in mice previously treated with reserpine (5 mg/kg intraperitoneally).

which produced a 50% mortality of mice given yohimbine) were 6.2, 3.6, 7.4 and 18 mg/kg, for imipramine, amitriptyline, chlorpromazine and chlorprothixene respectively. It was noticeable that even high doses of chlorpromazine and chlorprothixene, which produced profound sedation, still increased the yohimbine effects.

Increase in toxicity of yohimbine caused by other drugs

A variety of drugs was tested to see whether they acted like imipramine in increasing the toxicity of yohimbine. Four types of drug were investigated in particular since they were known to act on central or peripheral autonomic nervous mechanisms. The drugs are listed in Table 2, with their approximate ED50 values.

(a) *Central stimulants affecting adrenergic mechanisms.* Amphetamine, ephedrine and methyl phenidate strongly increased the toxicity of yohimbine. This was to

TABLE 2

ED50 VALUE, ROUTE AND TIME OF ADMINISTRATION OF DRUGS INCREASING THE TOXICITY OF YOHIMBINE IN MICE

ED50=the dose producing a 50% mortality of mice injected subcutaneously with yohimbine hydrochloride (20 mg/kg), which alone killed none of fifty-one mice. s.c.=subcutaneous; LSD=lysergic acid diethylamide; Aprobit=1-(10-phenothiazinyl methyl)ethyl-2-hydroxy-ethyldimethylammonium chloride

Drug	Route	Time (hr)	No. of mice	Approximate ED50 (mg/kg)
Imipramine-like drugs				
Imipramine HCl	Oral	1	245	6
Amitriptyline HCl	Oral	1	80	4
Chlorprothixene HCl	Oral	1	75	18
Phenothiazine tranquillizers				
Chlorpromazine HCl	Oral	1	90	7
Promazine HCl	Oral	1	50	9
Perphenazine	Oral	1	25	50
Monoamine oxidase inhibitors				
Iproniazid PO_4	Oral	1	35	200
Nialamide	Oral	1	40	9
Pargyline HCl	Oral	1	30	80
Phenelzine HCl	Oral	1	30	20
Pheniprazine HCl	Oral	1	30	20
Tranylcypromine SO_4	Oral	1	40	4
Adrenergic stimulants				
dl-Amphetamine SO_4	Oral	1	50	2
Cocaine HCl	s.c.	0·25	50	15
Ephedrine HCl	Oral	1	45	4
Methyl phenidate HCl	Oral	1	50	15
α-Receptor blocking drugs				
Dibenamine	s.c.	1	25	50
Dihydroergotamine methane sulphonate	s.c.	1	20	>20
Tolazoline HCl	s.c.	1	60	27
Phentolamine methane sulphonate	s.c.	1	40	46
Phenoxybenzamine	s.c.	1	30	33
β-Receptor blocking drugs				
Dichloroisoprenaline HCl	s.c.	1·5	20	>50
Pronethalol HCl	s.c.	1·5	20	>40
Miscellaneous drugs				
Atropine methyl NO_3	s.c.	1	30	15
Atropine SO_4	Oral	1	30	40
Bretylium tosylate	s.c.	1	25	>40
Bromo-LSD	s.c.	1	10	>10
Guanethidine SO_4	s.c.	1	20	>50
LSD	s.c.	1	20	>10
Mepyramine maleate	Oral	1	30	>50
Morphine HCl	Oral	1	30	>80
Promethazine HCl	Oral	1	20	>50
Aprobit	s.c.	1	35	16

be expected simply from their action in increasing the motility of the mice, thereby making them more likely to precipitate convulsions or trigger them off in other animals, but significant potentiation was observed at dose-levels slightly below those causing increased activity. Cocaine acted similarly.

(b) *Adrenergic blocking agents.* Phenoxybenzamine, dibenamine, phentolamine, tolazoline, dihydroergotamine and bretylium, given in fairly high doses, each increased the toxicity of yohimbine. Guanethidine gave a marginal increase. Reserpine failed to do so when given 0, 1, 4 or 24 hr before yohimbine. Dichloroisoprenaline increased the toxicity of yohimbine slightly, whereas the more specific drug for blocking β-receptors, pronethalol (Black & Stephenson, 1962), was inactive in sub-toxic doses.

(c) *Monoamine oxidase inhibitors.* Nialamide, iproniazid, pheniprazine, phenelzine, pargyline (*N*-benzyl-*N*-methylprop-2-ynylamine) and tranylcypromine increased the toxicity of yohimbine only within a few hours of administration. They failed to do so 24 hr later, even though monoamine oxidase inhibition by these compounds is known to last for several days and mice so treated displayed hypermotility and hyperthermia on injection of dopa (dihydroxyphenylalanine, 50 mg/kg) and 5-hydroxytryptophan (25 mg/kg) 24 hr after administration of the inhibitor.

(d) *Antagonists of acetylcholine, histamine and 5-hydroxytryptamine.* In moderate to high doses (10 to 50 mg/kg) both atropine and atropine methyl nitrate increased the toxicity of yohimbine to some extent. After effective doses, mice were usually alert and, after atropine, somewhat hyperactive. Of the antihistamine agents tested, promethazine failed to increase the toxicity of yohimbine and mepyramine did so only at high doses, whereas the quaternary compound, 1-(10-phenothiazinylmethyl)-ethyl-2-hydroxy-ethyldimethyl ammonium chloride (Aprobit), was more active. Lysergic acid diethylamide and, to a lesser degree, its bromo-derivative increased yohimbine toxicity only slightly.

Reduction of the combined toxicity of yohimbine and imipramine

A number of drugs were tested for their possible reducing effect upon the increase in toxicity of yohimbine (20 mg/kg) caused by imipramine (20 mg/kg) (Table 3).

Drugs capable of reducing or abolishing this action of imipramine fell into three classes:

(i) *Reserpine-like compounds.* Reserpine and syrosingopine (the latter in a dose which did not produce appreciable sedation) markedly reduced the toxicity of yohimbine plus imipramine (Fig. 1). Reserpine had less effect on the chlorpromazine and chlorprothixene action (Fig. 2). High doses of guanethidine were partially effective. Tetrabenazine, at a dose producing profound sedation, did not reduce the toxicity.

(ii) *Ganglion-blocking drugs.* All three ganglion-blocking drugs tested reduced the toxicity, chlorisondamine and pempidine to a marked degree and pentolinium partially.

(iii) *Antagonists for sympathetic β-receptors.* Agents which acted predominantly by blocking α-receptors (Ahlquist, 1948) increased the toxicity of yohimbine. The

TABLE 3

DRUGS REDUCING THE INCREASE IN THE TOXICITY OF YOHIMBINE CAUSED BY IMIPRAMINE

Imipramine hydrochloride (20 mg/kg orally) was given 1 hr before yohimbine hydrochloride (20 mg/kg subcutaneously); this combination alone killed 89% of mice tested. i.p.=intraperitoneal; s.c.=subcutaneous; LSD=lysergic acid diethylamide

	Drug	Dose (mg/kg)	Route	Time before yohimbine (hr)	*P*
A.	*Drugs reducing the increase in toxicity*				
	(*a*) *Marked* (>75%) *reduction in mortality*				
	Reserpine	5	i.p.	0–24	0·001
	Syrosingopine	0·5	i.p.	48, 30, 24 and 6	
		1·0	i.p.	24 and 6	<0·001
	Pronethalol HCl	40	s.c.	1·5	0·001
	Chlorisondamine Cl	10	s.c.	1	<0·001
	Pempidine tartrate	20	s.c.	1	0·002
	(*b*) *Partial* (40 *to* 75%) *reduction in mortality*				
	Guanethidine SO_4	25	s.c.	48, 30, 24 and 6	0·001
	Pentolinium tartrate	50	s.c.	1	<0·001
B.	*Inactive drugs* ($P>0·05$)				
	Dihydroergotamine methanesulphonate	5	s.c.	1·5	
	LSD	2·5–5	s.c.	1·5	
	Bromo-LSD	5	s.c.	1·5	
	Tetrabenazine	50–100	i.p.	3	
	α-Methyldopa	400	i.p.	4	
	Allobarbitone and urethane	27+615	i.p.	0·25	
	Hexobarbitone Na	100	i.p.	5 min	
	Meprobamate	100	Oral	0·5	
	Pentobarbitone Na	60	i.p.	0	
	Phenobarbitone Na	40–100	Oral	0·5	
	Urethane	750+500	i.p.	5+2	

β-blocking drug, dichloroisoprenaline, acted similarly, though to a lesser degree, but the newer agent pronethalol did not, and it significantly *reduced* the increase in toxicity of yohimbine induced by imipramine.

Drugs believed to interfere with synthesis or release of transmitters at sympathetic nerve-endings, such as α-methyldopa and bretylium, and drugs antagonizing the effects of 5-hydroxytryptamine, such as lysergic acid diethylamide and its bromo-derivative, failed to reduce the imipramine effect. Hypnotic agents (pentobarbitone and the allobarbitone-urethane mixture) in anaesthetic dosage were similarly ineffective, as was hexobarbitone. Phenobarbitone reduced the toxicity to a slight but not significant degree ($P=0.07$).

Influence of adrenalectomy

The reduction of the increase in toxicity of yohimbine induced by imipramine in response to ganglion-blocking agents and to reserpine and its analogue syrosingopine, which has been reported by Orlans, Finger & Brodie (1960) to deplete peripheral organs but not the brain of catechol amines, coupled with the failure of tetrabenazine to act similarly, prompted an investigation of the effect of adrenalectomy on the synergism of imipramine and yohimbine.

Adrenalectomy caused a highly significant reduction in the mortality due to this imipramine-yohimbine combination from 11 out of 15 of the untreated to 2 out of 15 of the operated animals.

Reduction of the toxicity of a high dose of yohimbine by various drugs

A number of drugs were tested for their ability to reduce the toxicity of a high dose of yohimbine (60 mg/kg) (Table 4). This dose alone killed 76% of mice injected.

TABLE 4

DRUG-TREATMENTS INEFFECTIVE IN REDUCING SIGNIFICANTLY THE TOXICITY OF YOHIMBINE HYDROCHLORIDE

Yohimbine (60 mg/kg) alone killed 76% of control mice. i.p.=intraperitoneal; s.c.=subcutaneous

Treatment	Dose (mg/kg)	Route	Time before yohimbine (hr)
Allobarbitone+urethane	27+615	i.p.	0·25
Chlorisondamine Cl	10	s.c.	1
Hyoscine HBr	10	s.c.	1
Meprobamate	100	Oral	0·5
Pronethalol HCl	40	s.c.	1·5
Pentobarbitone Na	60	i.p.	0
Phenobarbitone Na	40 or 100	Oral	0·25 or 0·5
Reserpine	5	i.p.	4 or 24
Reserpine+	5+	i.p.	24
α-methyldopa	400	i.p.	3

In contrast to its effect upon the toxicity caused by a low dose of yohimbine given with imipramine, reserpine failed to reduce the toxicity of the high dose of yohimbine alone. Combined treatment of the mice with reserpine and a dopa-decarboxylase inhibitor, α-methyldopa, was similarly ineffective in reducing the toxicity, as were various hypnotic, anaesthetic and anti-convulsant agents, hyoscine and a ganglion-blocking drug.

It appeared likely that the immediate cause of death after this high dose of yohimbine was respiratory depression (Strubell, 1906).

Effect of yohimbine and various other treatments on tissue amine levels

Evidence indicated that yohimbine produced a sympathetic discharge, at least from peripheral sites. To ascertain whether catechol amine liberation could be demonstrated from the brain and the adrenal glands, or only from the latter, the catechol amine contents of these organs were estimated in mice which had received yohimbine or various other drugs. The amounts of 5-hydroxytryptamine in their brains were also determined (Table 5).

(a) *Adrenal glands.* The standard dose of yohimbine used (20 mg/kg) reduced the total catechol amine content by 12% ; the reduction was significant at the 5% level. This effect occurred 40 min after injection and no further depletion was noted after another 40 min. Prior treatment of mice with imipramine, which alone did not affect the adrenal amine content, increased the depletion only slightly. A higher

TABLE 5

TOTAL CATECHOL AMINE CONTENT OF ADRENAL GLANDS AND NORADRENALINE AND 5-HYDROXYTRYPTAMINE CONTENTS OF BRAIN OF MICE AFTER VARIOUS DRUG-TREATMENTS

* Difference from saline-treated mice, $P<0{\cdot}05$. i.p.=intraperitoneal; s.c.=subcutaneous. Contents are means with standard errors. 5-HT=5-hydroxytryptamine. Total catechol amines are expressed as adrenaline. For determination of brain amine content, brains from four mice were pooled

				Brain content			Adrenal content	
Treatment	Dose (mg/kg)	Route	Time (hr)	No. of mice	Noradrenaline (μg/g)	5-HT (μg/g)	No. of mice	Total catechol amines (μg)
Saline	0·2 ml.	s.c.	0·5	44	0·49±0·05	0·58±0·02	45	7·59±0·30
Yohimbine HCl	20	s.c.	0·67	32	0·36±0·02*		30	6·72±0·54*
	60	s.c.	1·33	20	0·12±0·02*		20	6·06±0·49*
Imipramine HCl	20	Oral	1·67	24	0·50±0·05	0·63±0·06	22	7·37±0·82
Imipramine HCl+	20+	Oral	1·67	24	0·25±0·03*		27	6·45±0·51*
yohimbine HCl	20	s.c.	0·67					
Reserpine	5	i.p.	4				10	7·03±0·57
			20	24	0·04±0·002*	0·29±0·07*	28	5·43±0·93*
Tetrabenazine	100	i.p.	3	24	0·07±0·007*		24	6·33±0·41*
Syrosingopine	1+1	i.p.	24+6	28	0·50±0·02	0·65±0·04	20	6·84±0·45

dose of yohimbine (corresponding roughly to an LD80) caused a mean reduction of adrenal catechol amines of 20% ($P=0.01$).

When the effects of reserpine and its analogues were examined on mouse adrenal glands, however, it became apparent that the adrenal glands of this species were relatively resistant to depletion by these agents. Reserpine (5 mg/kg, intraperitoneally) produced only an insignificant reduction of 7% within 4 hr of injection, and 28% ($P=0.01$) by 20 hr. Tetrabenazine caused a significant depletion, but syrosingopine in doses used here did not.

(b) *Brain.* In contrast to the catechol amines of the adrenal glands, the amount of noradrenaline in the brain was readily reduced by various agents. Yohimbine by itself caused a significant reduction of 27% ($P<0.001$) and of 49% when given after imipramine. Imipramine itself did not significantly alter the brain noradrenaline content. In agreement with results of other workers, it was shown that reserpine and tetrabenazine produced depletions of 92% and 85% respectively, whereas syrosingopine was without effect on brain noradrenaline levels.

Determination of the 5-hydroxytryptamine content of the brains of mice treated with yohimbine was rendered impossible by the presence in the extracts of a substance which caused interference in the fluorimetric assay. The substance contributed to the fluorescence measured at the wavelength of 550 mμ (via an interference filter with half-band width of 18 mμ) upon activation by ultra-violet light (295 mμ), and was probably yohimbine itself. A similar but not identical type of interference was observed in extracts of brains from mice treated with tetrabenazine.

Imipramine and syrosingopine did not reduce brain 5-hydroxytryptamine levels; reserpine caused a depletion of 50% ($P<0.01$).

Effect of yohimbine on brain monoamine oxidase activity

Yohimbine hydrochloride (60 mg/kg) did not alter significantly the monoamine oxidase activity of the brains of mice injected with the drug 1 to 2 hr beforehand. Seven of the twelve mice injected were dead by this time.

The effect of yohimbine, with and without previous treatment with imipramine, on the heart-rate of anaesthetized rats

The effect of yohimbine was examined also upon the heart-rate of rats anaesthetized with a mixture of allobarbitone and urethane, without and with previous treatment with atropine and imipramine. The time-courses for the changes in mean heart-rates of rats given these treatments are shown in Fig. 3. Yohimbine (20 mg/kg) produced by itself a bradycardia, which was apparently mediated via vagal mechanisms, since it was not seen in rats previously treated with atropine 20 mg/kg. In animals treated with imipramine (20 mg/kg) subcutaneously 2 hr beforehand, however, injection of yohimbine produced a marked and long-lasting tachycardia. This tachycardia was slow in onset, and the heart-rates of individual rats often showed a short-lasting initial slowing during the first hour after yohimbine injection, similar to, but shorter than, that seen in control animals given yohimbine

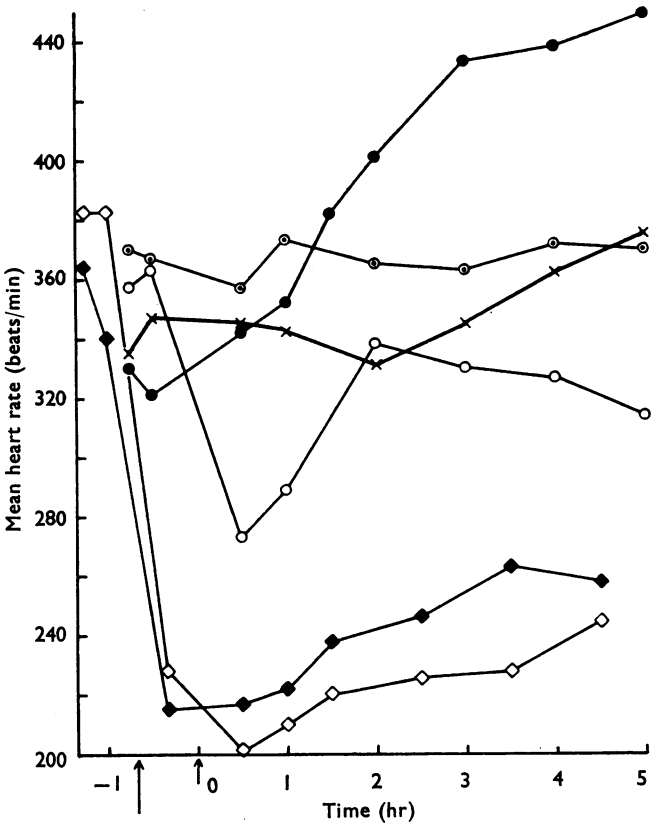


Fig. 3. The interaction of various drugs with yohimbine hydrochloride (20 mg/kg) on the heart-rate of rats anaesthetized with a mixture of allobarbitone and urethane. Yohimbine or saline was injected at 0 hr (second arrow); pronethalol hydrochloride (15 mg/kg) or saline was injected at the first arrow. Some rats were previously treated with imipramine hydrochloride (20 mg/kg) or with atropine sulphate (20 mg/kg) at −2 hr. All injections were subcutaneous. × —— × saline only; ○ —— ○ yohimbine only; ⊙——⊙ atropine and yohimbine; ● —— ● imipramine and yohimbine; ◇——◇ pronethalol and yohimbine; ◆——◆ imipramine, pronethalol and yohimbine.

only. The sympathetic β-receptor blocking agent pronethalol, which itself caused a precipitous and long-lasting fall in heart rate, abolished this tachycardia in rats treated with imipramine.

DISCUSSION

The clinical observation of Holmberg & Gershon (1961) that the autonomic and psychic effects of yohimbine were markedly accentuated in patients receiving treatment with the anti-depressant drug imipramine has prompted an investigation into the stimulatory actions of yohimbine in mice and their augmentation by imipramine. In this species yohimbine appears to exaggerate the normal "alarm"

reactions (for example alerting, apprehension and flight) occasioned by external stimuli, so that, when disturbed, the mice exhibit short bursts of intense hyperactivity which often terminate in clonic convulsions. This occurs particularly if the mice have been treated with imipramine; in animals so-treated, a dose of yohimbine which is not lethal by itself may cause death. The excitatory effect of yohimbine differs from that of amphetamine by the absence of any continuous hyperactivity; also, the toxicity of yohimbine, unlike that of amphetamine, is not influenced by grouping of the mice.

Since some of the symptoms of treatment with yohimbine suggest central sympathetic stimulation and since imipramine is known to increase the effects of catechol amines (Sigg, 1959; Schaeppi, 1960), probably by interfering with their inactivation by binding in storage sites, other drugs known to interfere with the tissue binding of amines (Dengler, Spiegel & Titus, 1961; Hertting, Axelrod & Whitby, 1961; Davey, Farmer & Reinert, 1963) were tested. Many such compounds increased the toxicity of yohimbine, even though they possessed different pharmacological actions in other ways. For instance, stimulants of adrenergic mechanisms, such as amphetamine, ephedrine, cocaine and methyl phenidate, adrenaline antagonists such as phenoxybenzamine, dibenamine, phentolamine and tolazoline, and inhibitors of monoamine oxidase such as nialamide, tranylcypromine and phenelzine all increased the toxicity of yohimbine. Further evidence which supported the implication of catechol amines or 5-hydroxytryptamine in the synergism of yohimbine and imipramine was provided by the finding that their toxicity was greatly reduced in mice given reserpine, which presumably reduced the amounts of available amine in the tissues. Reserpine has been reported to reduce the autonomic and psychic effects of yohimbine in man (Ingram, 1962).

Evidence at this point in the investigation indicated that stimulation of one or more of three types of amine receptor was involved—sympathetic α- or β-receptors (Ahlquist, 1948) or tryptamine receptors. Since yohimbine itself possesses α-receptor blocking activity (Barry, 1937; Yonkman, Stilwell & Jeremias, 1944) and since other α-blocking agents potentiate the toxicity of yohimbine, stimulation of α-receptors seemed improbable. Similarly, since the 5-hydroxytryptamine antagonists lysergic acid diethylamide and its bromo-derivative failed to reduce the toxicity of yohimbine and imipramine, tryptamine receptors appeared unlikely to be affected. Confirmation of the role of sympathetic β-receptors in this interaction was provided by two observations. Firstly, the β-blocking agent pronethalol (Black & Stephenson, 1962) significantly reduced the combined toxicity of yohimbine and imipramine. Dichloroisoprenaline failed to do so, and actually increased toxicity of yohimbine to some extent, presumably because of its agonist properties (Dresel, 1960; Black & Stephenson, 1962). Secondly it was shown in anaesthetized rats that yohimbine, which caused a bradycardia by itself, produced a marked and long-lasting tachycardia in animals previously treated with imipramine. This tachycardia was abolished by pronethalol and was presumably due to stimulation of β-receptors in the heart.

The source of the catechol amines involved in this stimulation of β-receptors was next investigated. Since the toxicity of yohimbine with imipramine was significantly

reduced by reserpine and by syrosingopine (Orlans *et al.*, 1960) but not by tetrabenazine (Quinn, Shore & Brodie, 1959), peripheral rather than central sources seemed likely. The results obtained with ganglion-blocking agents and adrenalectomy, which significantly reduced the toxicity of yohimbine with imipramine, supported this view. Since yohimbine appeared to lack nicotine-like properties, it seemed likely to be acting by stimulation of the hypothalamic-adrenal axis, an action similar to that shown by Vogt (1954) for morphine and picrotoxin, leading to release of catechol amines from both the hypothalamus and the adrenal glands.

This hypothesis was examined by determining catechol amine and 5-hydroxytryptamine levels in the brain and adrenal glands of mice treated with yohimbine and other drugs. Yohimbine significantly reduced total brain noradrenaline, the degree being somewhat increased in animals treated with imipramine. The total catechol amine content of the adrenal glands was reduced to a slight but just significant extent. Reserpine, however, depleted mouse adrenal glands of catechol amines by only 30%, even though it reduced brain levels by over 90% and has been shown to reduce the adrenal catechol amines more markedly in other species (Kroneberg & Schümann, 1957).

Syrosingopine, in the dose used, failed to cause a significant depletion of mouse adrenal catechol amines even though it reduced the toxicity of yohimbine and imipramine. It may be that any marked depletion of adrenal catechol amines by drugs acting on the hypothalamic–adrenal axis is difficult to prove in mice by reason of the high rate of amine synthesis in these organs. Holland & Schümann (1956) and Kroneberg & Schümann (1959) have shown that the rate of amine synthesis in adrenal glands is dependent upon the degree of nervous activity, being supranormal in stimulated glands and subnormal in denervated glands. After treatment with a drug such as yohimbine, which produces a central sympathetic nervous discharge, amine synthesis in the adrenal glands would no doubt be accelerated.

The evidence appears to support the following hypothesis of the mechanism of the toxic interaction of yohimbine and imipramine. Yohimbine stimulates sympathetic centres in the brain, leading to an increased sympathetic discharge both in the hypothalamus and in peripheral organs, particularly the adrenal glands. The inactivation of the catechol amines thus released is depressed by imipramine, which reduces the binding of the amine at storage sites in the tissues, and the amines therefore are able to reach receptors for adrenergic transmission in appreciable amounts. Since the α-receptors are blocked by yohimbine, stimulation occurs particularly at β-receptors, especially those in the heart. Death may then occur, possibly from ventricular fibrillation.

The finding that yohimbine, like reserpine, depletes the brain and adrenal glands of catechol amines, and the failure of reserpine to cause any significant potentiation of toxicity of yohimbine when administered either at the same time or only shortly before yohimbine, despite its known action in blocking tissue uptake of noradrenaline (Hertting *et al.*, 1961), may be interpreted as a possible indication that the two drugs act at the same sites. Some degree of competitive inhibition may be suggested, since reserpine markedly reduces the potentiation of toxicity of yohimbine by imipramine even when injected at the same time as the yohimbine (that is too late for it to cause

appreciable depletion of tissue amines before the yohimbine acts). The tachycardia seen in rats previously treated with imipramine and then injected with yohimbine is similar in rate of onset and degree to that seen in rats treated with imipramine and then given reserpine (Quinton, unpublished). Furthermore, yohimbine appears to deplete adrenal gland catechol amines in the same proportion to its depletion of brain noradrenaline as does reserpine, although the latter (at the doses used in this work) is considerably more effective. Reserpine acts also at sites of 5-hydroxytryptamine storage, causing a marked depletion of this amine ; it has been postulated that the tranquillizing and sedative actions of reserpine (which are not apparent in the case of yohimbine) run parallel to its action upon brain levels of 5-hydroxytryptamine, not of catechol amines (Costa, Gessa, Hirsch, Kuntzman & Brodie, 1962). In the present work, it has not been possible to determine the effect of yohimbine upon brain 5-hydroxytryptamine levels.

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