Br. J. Pharmac. Chemother. (1967), **30**, 541–553.

FREQUENCY-DEPENDENT POTENTIATION BY VARIOUS DRUGS OF THE CHRONOTROPIC RESPONSE OF ISOLATED CAT ATRIA TO SYMPATHETIC NERVE STIMULATION*

BY

C. N. GILLIS† AND F. H. SCHNEIDER‡

From the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut, U.S.A.

(*Received February* 17, 1967)

The importance of its uptake into sympathetic nerve endings as a mechanism for terminating the pharmacological actions of injected noradrenaline (NA) is supported by the work of many investigators (Huković & Muscholl, 1962; Thoenen, Hürlimann & Haefely, 1964a; Trendelenburg, 1965; see also Trendelenburg, 1963, 1966). The ability of sympathetic nerve fibres to accumulate, store and release exogenous NA suggests that the endogenously liberated amine can also be bound (and therefore functionally inactivated) by the sympathetic nerve endings after its release by nerve stimulation. However, the relation between such re-uptake and the frequency of stimulation, particularly within the physiological range of frequencies, has received relatively little consideration.

Brown and his co-workers (see Brown, 1960, 1965) reported that the release of NA into the venous outflow from the cat spleen was greater with sympathetic nerve stimulation (SNS) at 30/sec than at 10/sec. In the presence of phenoxybenzamine, liberation of NA was increased approximately five- to ten-fold with stimulation at 10/sec, but only slightly at 30/sec. Since phenoxybenzamine decreases re-uptake of released NA (Thoenen, Hürlimann & Haefely, 1964b), this observation indicates that, in the absence of the drug the magnitude of re-uptake at 10/sec is greater than at 30/sec. Haefely, Hürlimann & Thoenen (1964), demonstrated that cocaine-induced potentiation of cat nictitating membrane contractions, in response to sympathetic nerve stimulation, increased as the frequency of nerve stimulation was decreased from 3.3 to 0.1/sec. It was concluded that

* This study was aided by Public Health Service Grant H-7249 from the National Heart Institute, and by Grant G-65-46 from the Life Insurance Medical Research Fund. Part of this work was presented at the April, 1966, meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey.

† This work was done during the tenure (by C. N. G.) of an Established Investigatorship of the American Heart Association.

‡ This work represents partial fulfilment by F.H.S. of the requirements of the Ph.D. degree of Yale University. Predoctoral Fellow (Public Health Service Grant 5-T4-CA-5012). Present address: Department of Pharmacology, University of Colorado Medical Center, Denver, Colorado, U.S.A.

the ratio of NA inactivated to that liberated became progressively smaller as the stimulation frequency increased. A similar conclusion was reached by Gillis, Schneider, Van Orden & Giarman (1966) on the basis of biochemical and microfluorometric evidence obtained with isolated cat atria. The possible physiological significance of a frequency-dependent process for terminating or controlling the action of neurally released NA prompted a detailed investigation of this phenomenon.

METHODS

Isolated atria

Isolated, sympathetically innervated cat atria were prepared as described previously (Chang & Rand, 1960; Gillis *et al.*, 1966). A Grass Model 4-S stimulator was used to deliver square-wave pulses of 1 or 2 msec duration, at a variety of voltages and frequencies, to the right cardio-accelerator nerve. The voltage/response relationship for each preparation was obtained at a frequency of 3 or 4/sec, and the voltage midway between threshold and maximum was selected for subsequent determination of the frequency/response relationship in that preparation. In each experiment equal numbers of stimuli were applied at all frequencies of stimulation, and sufficient stimuli were delivered to ensure production of the maximum response attainable at the particular voltage and frequency employed. Stimulation frequencies generally were less than 10/sec, since our primary interest lay in this, the physiological range (Folkow, 1952; Celander, 1954).

Enzyme assays

The method of McCamon (1961), as modified by Wurtman & Axelrod (1963), was used to measure monoamine oxidase (MAO). Catechol-O-methyl transferase (COMT) was assayed by the technique described by Axelrod & Tomchick (1958), except that tritiated noradrenaline (^{3}H-NA) was used as substrate in place of adrenaline. In both assays, activity was expressed as counts/min extracted from the assay mix into the organic solvent phase. A crude homogenate of cat atria prepared by homogenizing the tissue in a tapered ground glass homogenizer in 5 vol cold isotonic potassium chloride served as the source of MAO. The supernatant obtained by centrifugation of this homogenate at 78,000 × g (30 min) was used as the source of COMT. A blank value, obtained using heat-denatured enzyme in place of whole enzyme, was determined during each assay and was used for correction of all tissue enzyme values.

Accumulation of ^{3}H-NA by ventricle slices

A method similar to that described by Dengler, Spiegel & Titus (1961) was used. Slices of cat ventricle (75 to 125 mg) were prepared with a Stadie-Riggs microtome blade and were placed in 10 ml. previously oxygenated Krebs-bicarbonate solution at 37°. Fifteen minutes later ^{3}H-NA was added to give a concentration of 10 mμg base/ml. After incubation for an additional 45 min, the slices were removed, washed twice in cold isotonic saline, blotted dry, and homogenized in 3 ml. cold 0.4 M perchloric acid. Homogenates were allowed to stand in ice for 2 hr and were then centrifuged at 28,000 × *g* for 15 min. The tritium content of 0.1 ml. of the supernatant fluid was determined as described previously (Schneider & Gillis, 1965).

Drugs

The following drugs were used: cocaine hydrochloride, imipramine hydrochloride, isopropylnoradrenaline hydrochloride, methylphenidate, (±)-norepinephrine-7-^{3}H hydrochloride (specific activity, 6.2 c/mmole, supplied by The New England Nuclear Corporation), pheniprazine hydrochloride, phenoxybenzamine hydrochloride and tropolone. All concentrations are expresed as μg of the salt (with the exception of imipramine and tropolone, which are expressed in terms of the free bases) per ml. of the bathing fluid.

To test their effects on atria, drugs were added to Krebs solution in the reservoir supplying the tissue bath. After exposing preparations to drug-containing solutions, sufficient time was allowed for atrial rate to reach a constant level before proceeding with the experiment. Cocaine, methylphenidate

and imipramine were used in concentrations that increased the frequency of contraction in response to SNS, but which had no visible toxic effect on atria.

RESULTS

Response to nerve stimulation

Stimulation of the cardioaccelerator nerve with impulses of adequate voltage, duration and frequency, increased contraction rate, and either increased, decreased or failed to affect the force of contraction of atria. The variability of inotropic responses necessitated use of the chronotropic effect as the sole measure of the response to nerve stimulation. Reproducible chronotropic responses to repeated stimulation of the cardioaccelerator nerve were obtained if sufficient time elapsed between stimulation periods. Figure 1 shows that with either low (1.5 or 2/sec) or higher (6 or 7/sec) frequency stimulation, responses were more reproducible when 5 min, rather than 1 min, elapsed between stimu-

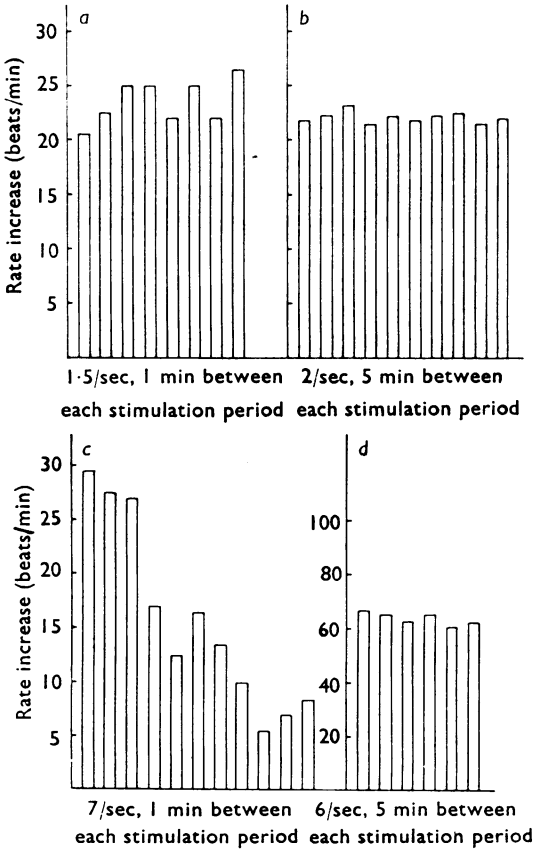

Fig. 1. Chronotropic responses to repeated periods of nerve stimulation in 4 different atrial preparations. Stimulation, 1 msec, submaximal voltage, for 60 sec. The bathing medium was changed by overflow immediately after each stimulation period. In (a) the prestimulation rates varied between 77 and 85 B/m; it was between 46 and 57 B/m in (b), between 131 and 137 B/m in (c) and between 107 and 112 B/m in (d).

lation periods. Chronotropic responses gradually declined when frequency greater than 7/sec were used, regardless of the time between successive periods of stimulation.

The time required to reach a constant contraction rate after beginning stimulation depended upon the frequency (and voltage) of the stimulus; with frequencies between 0.5 and 5/sec the maximum response was attained within 30 to 60 sec. After stimulation the decay of the response—that is, return of rate to the prestimulation level—took 1 to 3 min. Decay was most pronounced during the first 10 to 20 sec after stimulation (Fig. 4, control curve).

Frequency dependence of decay rate

Upon increasing the stimulation frequency (but at all frequencies delivering a constant number of stimuli), a slight delay appeared in the rapid decay in rate after stopping stimulation. Although only of the order of 5 to 10 sec, the delay was consistent and reproducible. Accordingly, the phenomenon was examined further by determining the decay rate after stimulation at a variety of frequencies, each applied for two different lengths of time. Figure 2 is a plot of the difference (beats/min) between the prestimulation (control) rate and that 30 sec after stopping stimulation against the length of time for which each frequency was applied. It is evident that when low frequency stimulation (1 or 2/sec) was used, increasing the total number of stimuli had no effect on the decay rate; at higher frequencies (6, 10 or 15/sec) however, the decay was delayed by lengthening the stimulation time. It must be emphasized that in every case the maximum rates

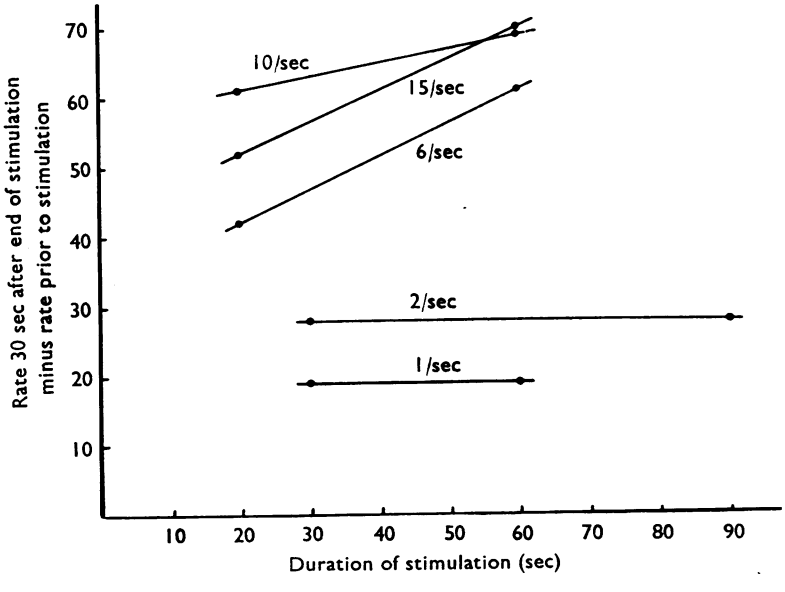

Fig. 2. The influence of the number of impulses, delivered at different frequencies, on the decay of the chronotropic response after cessation of nerve stimulation. Stimulation, 1 msec and submaximal voltage. The maximum rate attained at each frequency was the same regardless of the duration of stimulation.

attained at any one frequency were the same regardless of the length of time during which stimulation was applied. Results similar to those illustrated were obtained in each of 3 experiments.

Pharmacological studies

Cocaine

Frequency/response relationships were obtained in 6 preparations exposed to cocaine at concentrations of 2×10^{-6} g/ml. (n=5) and 3×10^{-6} g/ml. (n=1). A typical experiment is illustrated in Fig. 3. Responses are expressed both as the absolute increase (beats/min)

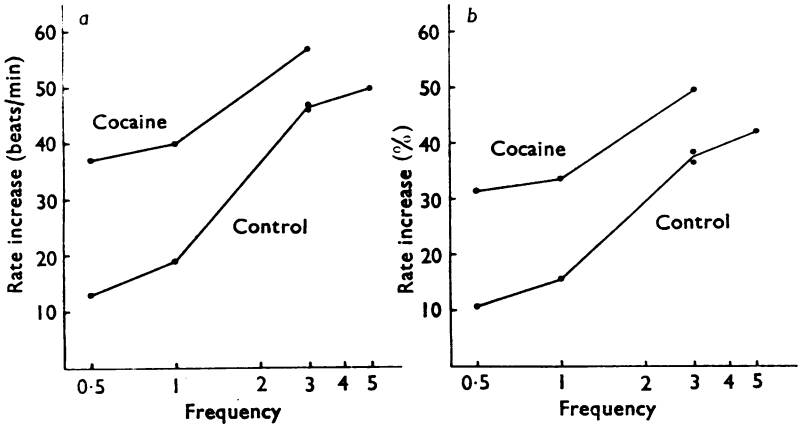

Fig. 3. Influence of cocaine on frequency/response relationships following sympathetic nerve stimulation. Stimulation ; submaximal voltage with 2 msec impulse duration. 300 stimuli delivered during each stimulation period. Cocaine, 2×10^{-6} g/ml.

in contraction rate (Fig. 3*a*) and as percent increase over the prestimulation rate (Fig. 3*b*). Similar results were obtained when responses were expressed as a % of the maximum rate attainable. In each experiment, cocaine-induced potentiation of the chronotropic responses to SNS increased in magnitude as the stimulation frequency decreased. This is indicated both by the decreased slope of the frequency/response line after cocaine (Fig. 3), and also by increasing values of the ratios of responses (increase in beats/min) after cocaine to those obtained before addition of the drug (Table 1).

In the presence of cocaine, the onset of the response was delayed 5 to 10 sec in 5 preparations, and the rapid phase of decay was prolonged 10 to 30 sec in all cases. Figure 4 shows the typical time-course of a response in the presence of cocaine, and illustrates the delay in both onset and decay produced by this drug.

Imipramine

Frequency/response relationships were obtained in 2 preparations both before and after imipramine ; in these preparations, the drug increased responses in a manner that was frequency dependent (Table 1). In 3 additional preparations imipramine was used in combination with either tropolone or pheniprazine (see below). In each of the 5 preparations, the onset and decay of the response was delayed by imipramine.

TABLE 1

EFFECT OF VARIOUS DRUGS ON CHRONOTROPIC RESPONSES OF THE ISOLATED CAT ATRIA TO SYMPATHETIC NERVE STIMULATION

Stimulation was performed at 1 msec impulse duration (voltage 50% of maximum) and usually consisted of 100 impulses, although in some experiments 150 impulses were delivered. The sensitization index is the ratio of responses (increase in beats/min) after the drug to those before

Drug	Concentration (g/ml.)	Preparations (no.)	Frequency (impulses/sec)	Frequency (times used)	Sensitization Index range	Sensitization Index average
Cocaine	2×10^{-6}	5	0·35	1	—	3·57
	3×10^{-6}	1	0·5	5	1·91–3·22	2·55
			0·75	1	—	1·85
			1	4	1·36–2·42	1·95
			1·5	3	1·56–1·71	1·65
			2	3	1·10–1·78	1·34
			3	4	1·14–1·41	1·23
			4	1	—	1·07
Imipramine	5×10^{-7}	1	0·5	2	2·89–5·12	4·01
	10^{-6}	1	1	2	2·17–2·34	2·26
			2	1	—	1·54
Methylphenidate	10^{-6}	5	0·5	5	2·17–6·57	3·80
			1	5	1·80–2·78	2·13
			1·5	4	1·49–2·63	2·07
			2	4	1·26–2·06	1·76
			3	1	—	1·72
Tropolone	$1·2\times10^{-5}$	1	0·5	2	0·56–1·18	0·87
	$1·2\times10^{-4}$	2	1	3	0·80–1·78	1·21
			1·5	3	0·80–1·19	1·02
			2	2	0·91–1·91	1·05
			2·5	1	—	0·86
			3	2	0·87–1·10	0·99

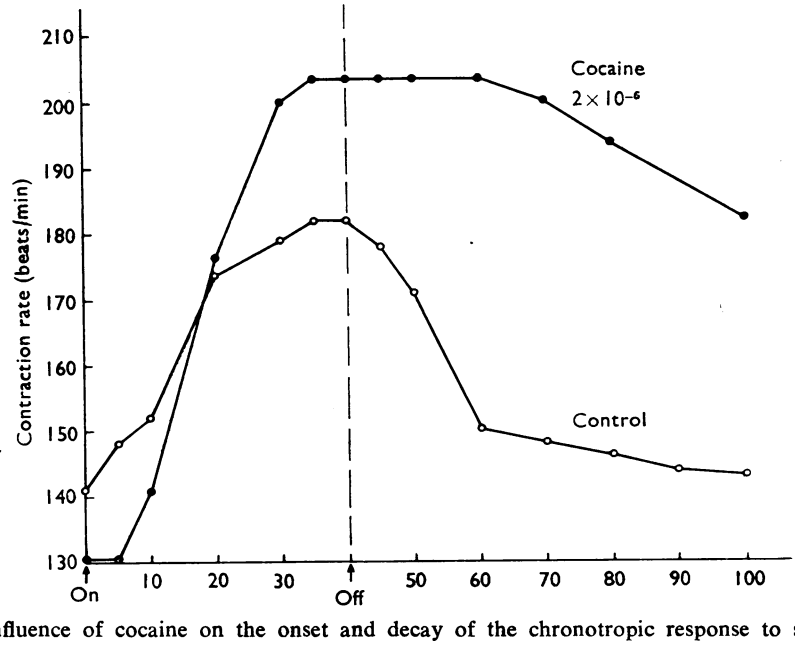

Fig. 4. Influence of cocaine on the onset and decay of the chronotropic response to sympathetic nerve stimulation. Stimulation ; submaximal voltage with 1 msec duration ; 60 stimuli given at 1.5/sec. Cocaine, 2×10^{-6} g/ml.

Phenoxybenzamine

Phenoxybenzamine had pronounced sympathomimetic effects on atria, and its use was usually associated with a high incidence of arrhythmic contraction patterns. Therefore a full frequency/response relationship in the presence of this drug was obtained in only 1 preparation. In this case, phenoxybenzamine (10^{-6} g/ml.) potentiated responses to SNS and the degree of potentiation was inversely related to frequency of stimulation. The ratio of response in the presence of phenoxybenzamine to those in its absence were 3.14 (0.5/sec), 2.80 (1/sec), 1.70 (2/sec) and 1.17 (4/sec). Both the onset and decay of responses were delayed by the drug.

Methylphenidate

In 5 preparations responses to submaximal SNS were potentiated by methylphenidate; potentiation was clearly frequency-dependent (Table 1). A delay (5 to 10 sec) in the onset and decay of the response was observed in the presence of methylphenidate.

The action of methylphenidate on the responses of atria to added NA and isopropylnoradrenaline was also examined. In 2 preparations, dose/response relationships were obtained for both amines before and after the addition of methylphenidate to the medium. The results of these experiments (Table 2), show that the responses to NA were potentiated while those to isoproterenol were unaffected.

TABLE 2

EFFECT OF METHYLPHENIDATE ON THE RATE INCREASES PRODUCED BY NORADRENALINE AND ISOPROPYLNORADRENALINE

Concentration of amine (mμg/ml.)	Rate increase (beats/min) before and after methylphenidate (10^{-6} g/ml.) preparation			
	1		2	
	Before	After	Before	After
Noradrenaline				
1·25	8	20	11	45
2·5	27	37	20	60
5	36	83		
Isopropylnoradrenaline				
0·1	16	19	15	13
0·15			26	22
0·2	24	23	31	26
0·5	77	56		

Tropolone

The COMT inhibitor tropolone was used at concentrations of 1.2×10^{-5} g/ml. (1 preparation) and 1.2×10^{-4} g/ml. (2 preparations). The onset and decay of the response were unaffected by the drug. Responses to SNS at various frequencies were either uninfluenced or slightly decreased by tropolone (Table 1); atrial COMT activity was reduced to 13% of control values by tropolone at a concentration of 1.2×10^{-5} g/ml. and was completely inhibited by 1.2×10^{-4} g tropolone/ml.

The effects of tropolone were also investigated on atria simultaneously exposed to imipramine (either 2 or 5×10^{-7} g/ml.) throughout the entire experiment. Although under these conditions the magnitude of responses to SNS were still uninfluenced by tropolone, the presence of both tropolone and imipramine prolonged the decay of the response (Table 3).

Pheniprazine

Because pheniprazine (2×10^{-6} g/ml.) frequently produced arrhythmias, frequency/response relationships were obtained in only 2 preparations exposed to this drug alone. In neither case did the drug increase the magnitude or alter the onset or decay of responses. Stimulation at 0.5 or 1/sec produced essentially normal responses, although at 1.5 or 2/sec they were depressed 20 to 50% by the drug. MAO activity was reduced to 38% of control. An additional preparation was exposed to imipramine (5×10^{-7} g/ml.) throughout the entire experiment; in this case also pheniprazine (10^{-6} g/ml.) had no effect on either the magnitude or the decay of the response (Table 3).

TABLE 3

EFFECTS OF TROPOLONE ($1{\cdot}2 \times 10^{-6}$ G/ML.) AND PHENIPRAZINE (10^{-6} G/ML.) ON THE DECAY OF THE CHRONOTROPIC RESPONSE IN THE SIMULTANEOUS PRESENCE OF IMIPRAMINE

Imipramine was present at concentrations of 2×10^{-7} g/ml. (preparation 1) or 5×10^{-7} g/ml. (preparations 2 and 3) throughout the experiments. Sympathetic nerve stimulation: 100 impulses of 1 msec duration at submaximal voltage. Where two values at any one frequency are given, stimulation at that frequency was applied twice during the experiment

Stimulation frequency (impulses/sec)	Contraction rate at the end of the stimulation period minus rate 60 sec later: Preparation 1 Control	1 Tropolone	2 Control	2 Tropolone	3 Control	3 Pheniprazine
0·5			7	3		
1	27	16	10	7	22	21·5
	28·5	14				25
1·5	31	16			30	27
					29·5	29
2	30·5	20	8·5	5·5	22·5	26
	27·5				23·5	24
3	30·5	18	16	3·5	29	24
	39				23	25

TABLE 4

EFFECT OF COCAINE, IMIPRAMINE AND METHYLPHENIDATE ON THE ACCUMULATION OF ^{3}H-NORADRENALINE BY SLICES OF CAT VENTRICLE AND ON THE ACTIVITY OF MONOAMINE OXIDASE AND CATECHOL-O-METHYL TRANSFERASE OF CAT ATRIA

The % inhibition of ^{3}H-NA retention was calculated according to Dengler *et al.* (1961). Per cent inhibition of enzyme activity in atrial homogenates was determined by dividing the activity in the presence, by that in the absence of drug and multiplying by 100. Both determinations were made with atria from the same animal

Drug	Concentration (g/ml.)	% inhibition: ^{3}H-NA retention by ventricle slices (mean±S.E.)	Atrial MAO	Atrial COMT
Cocaine	10^{-6}	52±6 (10)	0	0
	10^{-5}	79±8 (12)	0	5
	10^{-4}	—	0	4
Imipramine	10^{-6}	61±4 (10)	0	0
	10^{-5}	—	1	17
	10^{-4}	—	68	68
Methylphenidate	10^{-6}	50±5 (11)	0	0
	10^{-5}	—	0	8
	10^{-4}	—	30	37

Drug inhibition of NA accumulation in tissue slices

Table 4 shows the degree of drug-induced inhibition of ^{3}H-NA accumulation (in cat ventricle slices) at drug concentrations equal to or greater than those used in the experiments described above. Cocaine, imipramine and methylphenidate all reduced the amount of 3H-NA in the tissues at the end of the incubation period.

Drug inhibition of atrial MAO and COMT activity

Cocaine, imipramine and methylphenidate failed to affect enzyme activity at concentrations similar to or even 10 times greater than those that cause supersensitivity to SNS (Table 4).

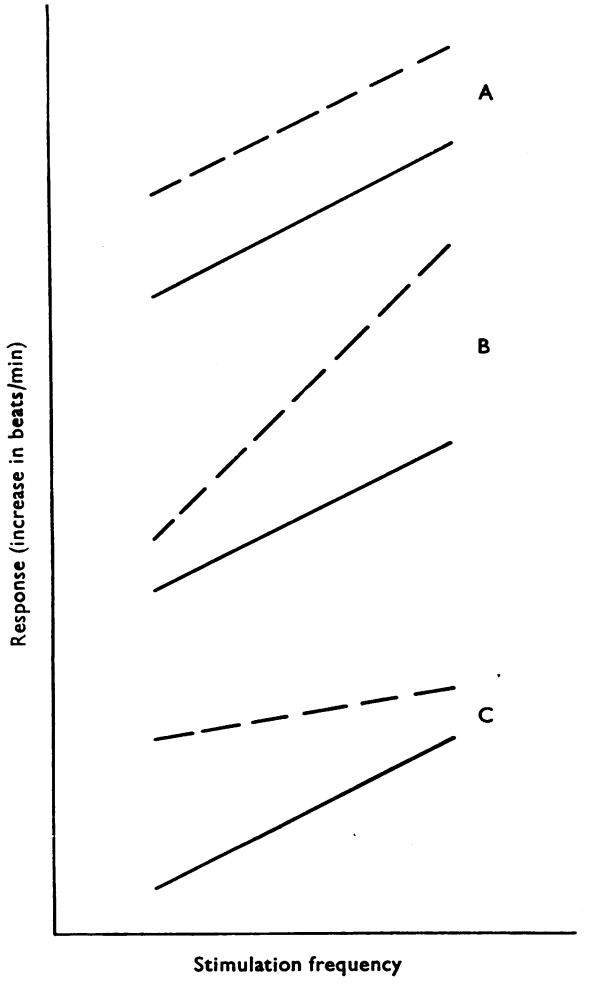

Fig. 5. Theoretical frequency/response curves illustrating three possible modes of drug-induced potentiation. Solid and broken lines represent relationships before and after drug treatment respectively.

DISCUSSION

In this study, drug-induced supersensitivity was evaluated by examining alterations in the shape of frequency/response curves after the addition of drugs. Before considering the drug-induced changes in frequency/response relationships that were observed it is necessary to examine alteration to such curves that might result from a number of theoretical mechanisms of potentiation. If the basis for supersensitivity were entirely nonspecific and independent of the frequency of nerve stimulation, the relationship might be altered in two ways. Should potentiation result from the addition of a constant increment to the response, frequency/response curves obtained during the control period would be parallel to those obtained after exposure to the drug, with the latter being above the control, as illustrated diagramatically in Fig. 5A. Alternatively, supersensitivity may result in a shift of the frequency/response curve such that the ratio of responses after sensitization to those before would be constant over the frequency range investigated. This situation might arise if a link in the events coupling nerve firing to the physiological response was sensitized by a constant factor ; the slope of the resulting curve would then be greater than than of control (Fig. 5B). On the other hand, potentiation that becomes quantitatively greater as the stimulation frequency is decreased would be expected to yield a frequency/response relationship with a slope less than that of the control (Fig. 5C). In this case, the ratio of responses after sensitization to those before would increase with decreasing frequency. It is obvious that these considerations are inapplicable either when maximum responses are reached, or when the frequency/response relationships deviate from linearity.

Comparison of control frequency-response curves with those obtained in the presence of drugs indicates potentiation that is greater at lower than at higher frequencies of stimulation. It can be seen in Fig. 3 that the slope of the curve following cocaine is generally less steep than that of the control, particularly between 0.5 and 1 c/s. The ratio of responses at each frequency in the presence of cocaine, methylphenidate or imipramine (Table 1), or phenoxybenzamine (see Results) to those in their absence decreased as the frequency was increased from 0.5 to 3 or 4 c/s. Such measures of vertical displacement, when applied to dose/response relationships have been criticised by Trendelenburg (1963). However, we believe the limitations Trendelenburg described are not applicable to frequency/response curves, since maximum responses were carefully avoided by using voltages and frequencies of stimulation that were shown, for each preparation, to be definitely submaximal. Furthermore, at the end of each experiment, stimulation of higher frequencies or voltage was used to demonstrate that responses greater than those seen during the experiment could in fact be produced. An equally important justification for our belief that " sensitization ratios," as used in this investigation, are valid estimates of potentiation is the following. Haefely, Hürlimann & Thoenen (1965) reported that stimulation of the splenic nerve released a constant amount of NA per stimulus from the cat spleen, previously exposed to cocaine and phenoxybenzamine. This was true with stimulation at frequencies between 1 and 4/sec ; at 0.5/sec the NA per impulse decreased slightly. If, as seems probable, the NA per stimulus released from atrial sympathetic nerve endings similarly remained constant regardless of the frequency of stimulation, then the increased response produced by drugs in this study must reflect only altered disposition of the amine after its release. This situation is, of course, quite different from that existing when graded doses of NA are used to assess dose/response relationships.

Neurally released NA is probably inactivated by diffusion of the amine from receptor areas, and by non-specific tissue binding, re-uptake into specific tissue-binding sites (nerve endings) and enzymatic destruction. Conceivably, the drugs employed here might potentiate responses to SNS by affecting any or all of these mechanisms, thus protecting released NA from inactivation. Although the effects of the drugs on the first two of these possibilities could not be determined, accumulation of ^{3}H-NA by cat heart slices was tested and found to be reduced by these agents (Table 4). Both cocaine and imipramine have previously been shown to reduce NA uptake by cat heart (Hertting, Axelrod & Whitby, 1961); this has not been previously shown for methylphenidate. Maxwell (1965) suggested that methylphenidate causes adrenergic supersensitivity in vascular tissue by a direct effect on adrenergic receptors. There seems little doubt however that methylphenidate-induced supersensitivity of atria to SNS is linked to its reducing the functional inactivation of the amine by tissue uptake. Thus the drug is as effective as cocaine in reducing ^{3}H-NA accumulation by ventricle slices (Table 4) and potentiates atrial responses to added NA, but not to isopropylnoradrenaline (Table 2), which is not taken up by the myocardium (Hertting, 1964). Also, if methylphenidate produced the conformational changes in adrenergic receptors proposed by Maxwell (1965), potentiation of atrial rate responses by the drug should be independent of stimulation frequency. The absence of any effect of cocaine, methylphenidate or imipramine on either MAO or COMT of atria (Table 4) precludes inhibition of these enzymes as the mechanism for the potentiation of responses to SNS. Support for this contention is also provided by the failure of tropolone or pheniprazine, in concentrations that markedly inhibited atrial COMT and MAO respectively, to produce potentiation. Therefore we conclude that the effective agents caused potentiation of atrial rate primarily by reducing re-uptake of neurally liberated sympathetic transmitter.

Since the transport of NA is a saturable process (Dengler, Michaelson, Spiegel & Titus, 1962; Iversen, 1963), it is likely that at higher frequencies of stimulation NA is liberated at a rate sufficient to saturate neural re-uptake, and therefore the concentration of unbound amine increases and leads to a greater response. At lower frequencies, on the other hand, re-uptake is probably able to keep pace with liberation, although a certain portion of neurally released NA will always reach the receptors. That levels of NA high enough to saturate the uptake process will be attained appears feasible upon consideration of the anatomical relationship of the sympathetic nerve endings to innervated tissue. Nerve endings are located in narrow grooves such that there are likely to be structural barriers capable of hampering the diffusion of amine from the site of its release (Richardson, 1962; Burnstock & Merrilless, 1964; Burnstock & Holman, 1966; Thaemert, 1966).

The action of cocaine (Fig. 4), methylphenidate and imipramine as well as stimulation with higher frequencies (Fig. 2), to delay the initial decrease in rate toward control levels at the end of stimulation is probably also linked to the production of a higher concentration of functionally active amine in the vicinity of receptors, thereby prolonging the response and increasing its magnitude. When re-uptake is blocked by imipramine, the COMT inhibitor tropolone delays still further the initial decay of the response (Table 4), suggesting that the enzyme may play a secondary role in the inactivation of NA. It is interesting that when imipramine was present, inhibition of MAO by pheniprazine did not

affect the decay, indicating that deamination appears to be of little importance in the inactivation of neurally released NA in cat atria; this finding supports the recent suggestion of Smith (1966).

SUMMARY

1. Isolated, sympathetically innervated cat atria were used to investigate the relationship between the frequency of nerve stimulation and the magnitude of potentiation of chronotropic responses in the presence of various drugs.

2. Preparations responded to nerve stimulation with an increase in rate that was both rapid in onset and of constant magnitude when repeated trains of stimuli were applied at frequencies less than 5/sec, provided sufficient time elapsed between successive periods of stimulation.

3. The normally rapid phase of the decay—that is, return of rate to prestimulation values—of the response after stopping stimulation was delayed by increasing the frequency of the stimulus.

4. Both the onset and decay of the rate response were delayed in the presence of cocaine, imipramine or methylphenidate, but were unchanged by pheniprazine or tropolone. In the simultaneous presence of imipramine, tropolone, but not pheniprazine, caused a further delay in the rapid part of the decay.

5. The magnitude of the response was potentiated by cocaine, imipramine or methylphenidate, but not by pheniprazine or tropolone.

6. The magnitude of potentiation produced by cocaine, imipramine or methylphenidate increased as the stimulus frequency decreased from 4/sec to 0.5/sec.

7. Cocaine, imipramine or methylphenidate reduced the accumulation of ^{3}H-NA in cat ventricle slices; these drugs failed to influence the monoamine oxidase or catechol-O-methyl transferase of cat atria at concentrations that caused supersensitivity to nerve stimulation.

8. It is concluded that the potentiation produced by these drugs results from inhibition of the re-uptake of neurally released NA, and that such re-uptake assumes greater quantitative significance as a mechanism of amine inactivation as the frequency of nerve stimulation decreases.

REFERENCES

AXELROD, J. & TOMCHICK, R. (1958). Enzymatic O-methylation of epinephrine and other catechols. *J. biol. Chem.*, **233**, 702–705.

BROWN, G. L. (1960). Release of sympathetic transmitter by nerve stimulation. *Ciba Foundation Symposium on Adrenergic Mechanisms*, pp. 116–124. Churchill, London.

BROWN, G. L. (1965). The release and fate of the transmitter liberated by adrenergic nerves. *Proc. Roy. Soc.*, **162**, B., 1–19.

BURNSTOCK, G. & HOLMAN, M. E. (1966). Junction potentials at adrenergic synapses. *Pharmac. Rev.* **18**, 481–493.

BURNSTOCK, G. & MERRILLESS, N. C. R. (1964). Structural and experimental studies on autonomic nerve endings in smooth muscle. In *Pharmacology of Smooth Muscle*, Ed. BÜLBRING, E. Pp. 1–17 Macmillan, New York.

CELANDER, O. (1954). The range of control exercised by the sympathicoadrenal system. *Acta physiol. scand.*, **32**, supp. 116.

CHANG, V. & RAND, M. J. (1960). Transmission failure in sympathetic nerve produced by hemicholinium. *Br. J. Pharmac. Chemother.*, **15**, 588–600.

DENGLER, H. J., MICHAELSON, I. A., SPIEGEL, H. E. & TITUS, E. O. (1962). The uptake of labelled norepinephrine by isolated brain and other tissues of the cat. *Int. J. Neuropharmac.*, **1**, 23–38.

DENGLER, H. J., SPIEGEL, H. E. & TITUS, E. O. (1961). Effect of drugs on uptake of isotopic norepinephrine by cat tissues. *Nature, Lond.*, **191**, 816–817.

FOLKOW, B. (1952). Impulse frequency in sympathetic vasomotor fibres correlated to the release and elimination of the transmitter. *Acta physiol. scand.*, **25**, 49–76.

GILLIS, C. N., SCHNEIDER, F. H., VAN ORDEN, L. S. & GIARMAN, N. J. (1966). Biochemical and microfluorometric studies of norepinephrine redistribution accompanying sympathetic nerve stimulation. *J. Pharmac. exp. Ther.*, **151**, 46–59.

HAEFELY, W., HÜRLIMANN, A. & THOENEN, H. (1964). A quantitative study of the effect of cocaine on the response of the cat nictitating membrane to nerve stimulation and to injected noradrenaline. *Br. J. Pharmac. Chemother.*, **22**, 5–21.

HAEFELY, W., HÜRLIMANN, A. & THOENEN, H. (1965). Relation between the rate of stimulation and the quantity of noradrenaline liberated from sympathetic nerve endings in the isolated perfused spleen of the cat. *J. Physiol.*, **181**, 48–58.

HERTTING, G. (1964). The fate of ^{3}H-iso-proterenol in the rat. *Biochem. Pharmac.*, **13**, 1119–1128.

HERTTING, G., AXELROD, J. & WHITBY, L. G. (1961). Effect of drugs on the uptake and metabolism of ^{3}H-norepinephrine. *J. Pharmac. exp. Ther.*, **134**, 146–153.

HUKOVIĆ, S. & MUSCHOLL, E. (1962). Die Noradrenalin-Abgabe aus dem Isolierten Keninchenherzen bei Sympathischer Nerveureizung und ihre Pharmakologische Beeinflussung. *Arch. exp. Path. Pharmak.*, **244**, 81–96.

IVERSEN, L. L. (1963). The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.*, **21**, 523–537.

MAXWELL, R. A. (1965). Concerning the mechanism of action of methylphenidate on the responses of rabbit vascular tissue to norepinephrine. *J. Pharmac. exp. Ther.*, **147**, 289–297.

MCCAMON, R. E. (1961). A micromethod for monoamine oxidase(s). *Fedn. Proc.*, **20**, 344.

RICHARDSON, K. C. (1962). The fine structure of autonomic nerve endings in smooth muscle of the rat vas deferens. *J. Anat.*, **96**, 427–442.

SCHNEIDER, F. H. & GILLIS, C. N. (1965). Catecholamine biosynthesis *in vivo*: an application of thin-layer chromatography. *Biochem. Pharmac.*, **14**, 623–626.

SMITH, C. B. (1966). The role of monoamine oxidase in the intraneuronal metabolism of norepinephrine released by indirectly acting sympathomimetic amines or by adrenergic nerve stimulation. *J. Pharmac. exp. Ther.*, **151**, 207–220.

THAEMERT, J. C. (1966). Ultrastructure interrelationships of nerve processes and smooth muscle cells in three dimensions. *J. Cell. Biol.*, **28**, 37–49.

THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1946a). Mode of action of imipramine and 5-(3′-methyl aminopropyliden)-dibenzo[a,e]cyclohepta[1,3,5]trien Hydrochloride (RO 4–6011), a new antidepressant drug, on peripheral adrenergic mechanisms. *J. Pharmac. exp. Ther.*, **144**, 405–414.

THOENEN, H., HÜRLIMANN, A. & HAEFELY, W. (1964b). Wirkungen von Phenoxybenzamin, Phentolamin und Azapetin auf Adrenergische Synapsen der Katzenmilz. *Helv. Physiol. Acta.*, **22**, 148–161.

TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225–276.

TRENDELENBURG, U. (1965). Supersensitivity by cocaine to dextrorotatory isomers of norepinephrine and epinephrine. *J. Pharmac. exp. Ther.*, **148**, 329–338.

TRENDELENBURG, U. (1966). Mechanisms of supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **18**, 629–640.

WURTMAN, R. J. & AXELROD, J. (1963). A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmac.*, **12**, 1439–1441.