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THE EFFECTS OF NIALAMIDE ON ADRENERGIC FUNCTIONS

BY

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Nialamide potentiated the pressor effects of noradrenaline in the pithed cat. In cats treated with reserpine and then pithed, it prevented the restoration of the pressor effects of tyramine by slow intravenous infusions of noradrenaline. Nialamide produced a gradual decline in the pressor effects of repeated injections of tyramine whilst the effects of tyramine on the nictitating membrane were potentiated. The development of tachyphylaxis to tyramine in the isolated guinea-pig heart was associated with a 40% reduction in the myocardial concentration of noradrenaline. The onset of tachyphylaxis to tyramine was more rapid when nialamide was either included in the perfusion fluid or administered *in vivo*. Prior treatment with nialamide increased threefold the myocardial concentration of noradrenaline; however, the development of tachyphylaxis to tyramine was associated with a proportionate fall in the myocardial concentration of noradrenaline. In five out of nine experiments the acute administration of nialamide increased the output of noradrenaline per stimulus from the isolated cross-perfused spleen of the cat when the stimulus frequency was 30 shocks/sec, but not when the frequency was 10 shocks/sec. When nialamide (20 mg/kg) was given subcutaneously to cats 20 hr before their spleens were isolated and perfused, there was a rapid fall-off in the contractions of the spleen in response to periods of nerve stimulation. The outputs of noradrenaline per stimulus were decreased at both frequencies of stimulation. Nialamide decreased the concentrations of noradrenaline in the myocardium and spleen of the cat. The hypothesis is proposed that nialamide diminishes the availability of transmitter, as a consequence of a decreased re-entry of noradrenaline into a storage site present in nerve endings, and that such a decrease in the availability of noradrenaline in hyperactive nerve pathways may account for the antihypertensive effects of monoamine oxidase inhibitors in man.

The antihypertensive effects of monoamine oxidase inhibitors have been confirmed by numerous clinical investigations (Sjoerdsma, 1960). However, the hypotensive effects in man were not predicted by pharmacological studies in experimental animals, and therefore further study with such animals seemed desirable.

It has been suggested that a failure of synaptic transmission in sympathetic ganglia may be responsible for the antihypertensive effects of monoamine oxidase inhibitors. Gertner (1961) reported that iproniazid, β-phenylisopropylhydrazine, β-phenylethylhydrazine, nialamide, tranylcypromine and harmine blocked synaptic transmission in the isolated perfused superior cervical ganglion of the cat. However, close-arterial injections of large doses of either nialamide or iproniazid, for periods of up to 4 hr, had no effect on the evoked ganglionic action potentials recorded from the superior cervical ganglion of the cat (Davey, Farmer & Reinert, 1962). Brodie & Kuntzman (1961) suggested that the antihypertensive action of the enzyme inhibitors

may result from an increased concentration of noradrenaline in sympathetic ganglia which antagonizes the effects of acetylcholine on the postsynaptic membrane. However, the evoked postganglionic action potentials of the isolated rabbit's superior cervical ganglion remained unaltered after 3 days of treatment of the rabbits with either nialamide, tranylcypromine or iproniazid, despite a twofold increase in the ganglionic concentration of noradrenaline (Reinert, 1963).

These results led us to conclude that the antihypertensive effects of monoamine oxidase inhibitors were not due to ganglionic blockade, and it was decided to examine the possibility that their site of action might be peripheral to the ganglionic synapse.

METHODS

Recording of the blood pressure and contractions of the nictitating membrane in pithed cats. Cats, male, female or neuter, have been used. Anaesthesia was induced with ether; a blunt rod was passed upward through the foramen magnum to destroy the medulla, pons, midbrain and thalamus and then downward to destroy the spinal cord. The animal was left for 1 hr to recover from the anaesthetic. Injections were made into the right femoral vein, and the mean arterial pressure was recorded with a mercury manometer from the left common carotid artery. An isotonic frontal writing lever of 7 g tension and with a 12-times magnification recorded the contractions of the right nictitating membrane. Heparin (500 units/kg) was used as anticoagulant. In ten experiments cats were treated with reserpine to deplete the catechol amine stores. A single dose of reserpine (5 mg/kg) was given subcutaneously 18 hr before the acute experiment.

Cross-perfusion of the isolated cat spleen. The method for perfusion of the spleen was that described by Abercrombie, Davies & Dwyer (1962), which is similar to that of Daly & Scott (1961) using dogs. The cats weighed from 2.0 to 4.5 kg; the smaller animals were selected to provide the spleen, and the larger animals were used to perfuse it.

Anaesthesia was induced with halothane in nitrous oxide and oxygen (3:1 v/v) and was maintained by the intravenous injection of 8.0 ml./kg of 1% chloralose in 0.9% saline.

The abdomen of a first cat was opened by a midline incision and the cat eviscerated from the terminal colon up to the mid-duodenum. The spleen was mobilized by ligation and section of all the vascular connexions with the omentum, pancreas and stomach. The splenic vein was prepared for cannulation, and the splenic nerves dissected from the splenic artery and tied. The splenic artery and vein were then tied, and cannulated toward the spleen with polyethylene tubing. The tissue remaining in the splenic pedicle was ligated and sectioned and the spleen removed from the abdomen and placed in a Perspex plethysmograph. Blood from a cannulated carotid artery of a second donor cat perfused the spleen through the splenic artery. Splenic venous blood was returned to the donor cat via a siliconed glass reservoir which emptied into an external jugular vein. The perfusion pressure was measured from the carotid cannula. The cat from which the spleen had been removed was bled, and the blood added to that in the perfusion circuit to minimize the effects of possible blood loss. The splenic nerves were placed on platinum wire electrodes (cathode distal) inside the plethysmograph, which was filled with liquid paraffin (previously equilibrated with saline) and maintained at 37° C.

Changes in the volume of the spleen were measured by means of a piston recorder connected to the plethysmograph. Heparin (500 units/kg) was used as anticoagulant.

The stimulus parameters and the techniques for collection and assay of splenic sympathin were those described by Brown & Gillespie (1957). A rectangular wave stimulator and dekatron gate were adjusted to deliver 200 supramaximal (20 V) rectangular pulses of 0.5 msec duration at frequencies of either 30 or 10 stimuli/sec. Periods of stimulation were repeated at 10 min intervals.

Blood was collected, into chilled silicone-coated graduated centrifuge tubes containing 1 mg heparin, during and for the first 20 sec after the period of nerve stimulation. Immediately after collection the volume of the sample was noted, the plasma and cells were separated by means of a refrigerated centrifuge, and the volume of plasma was noted. The plasma was pipetted off and was kept on ice until assayed. The sympathin content of the plasma was assayed against noradrenaline on the blood pressure of the pithed rat (Shipley & Tilden, 1947).

Isolated guinea-pig hearts. Isolated guinea-pig hearts were perfused with Krebs solution (g/l.): NaCl 7.1, KCl 0.35, $CaCl_2$ 0.28, $MgSO_4.7H_2O$ 0.28, $NaHCO_3$ 2.09, KH_2PO_4 0.16, glucose 1.0; equilibration continuously with 95% oxygen and 5% carbon dioxide, using Langendorff's method.

Determination of the tissue concentrations of noradrenaline. Tissue concentrations of noradrenaline were determined by the semi-automatic method of Merrills (1962). The method involves the homogenizing of the tissue in 0.3 M perchloric acid, adsorption on alumina of the noradrenaline contained in a neutralized aliquot of the perchloric acid extract, elution by means of appropriate *p*H adjustment, and finally fluorimetric estimation of noradrenaline in the eluate. The use of thioglycollic acid as the stabilizing agent makes this method specific for the estimation of noradrenaline. Nialamide, tyramine, dopamine, adrenaline, isoprenaline and 3,4-dihydroxyphenylalanine produced no interference with the estimation of noradrenaline, and the recovery of noradrenaline added to tissues was 90 to 100%.

Drugs. The following drugs were used: atropine sulphate, guanethidine sulphate, nialamide, noradrenaline bitartrate, reserpine and tyramine hydrochloride.

RESULTS

The effects of nialamide on the response of the blood pressure of the cat to noradrenaline

Single intravenous injections of nialamide (1 to 10 mg/kg) had no effect on the responses of the blood pressure to small doses of noradrenaline in pithed cats (Fig. 1). However, if the cats had been treated with reserpine (5 mg/kg subcutaneously) 18 hr before the acute experiment, then single intravenous injections of nialamide

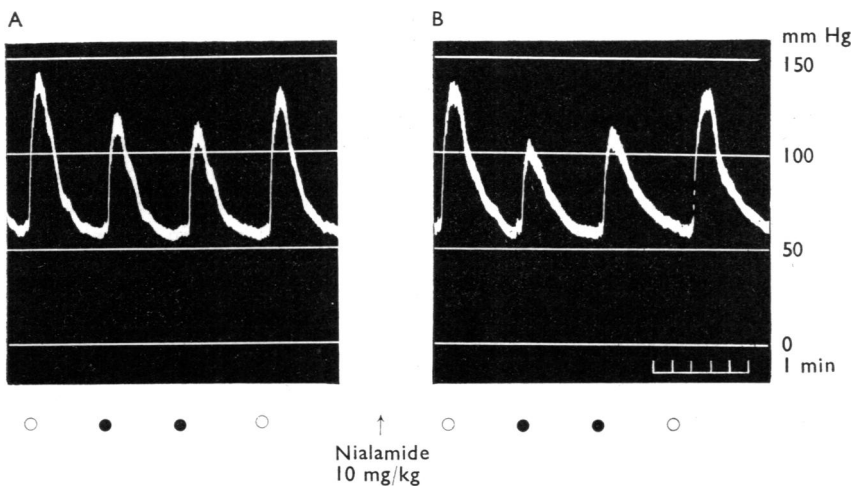


Fig. 1. Pithed cat. Blood pressure responses to intravenous injections of noradrenaline. Open circles: noradrenaline, 1.0 μg; closed circles: noradrenaline, 0.5 μg. Between A and B (10 min), nialamide, 10 mg/kg intravenously.

(1 to 10 mg/kg) potentiated the pressor effects of noradrenaline (Fig. 2). This suggested that nialamide might interfere with the penetration of noradrenaline into intracellular sites when the endogenous tissue stores of noradrenaline had been depleted, and thus prolong its presence in an effective concentration at receptor sites. In cats which had not been treated with reserpine the binding sites, because

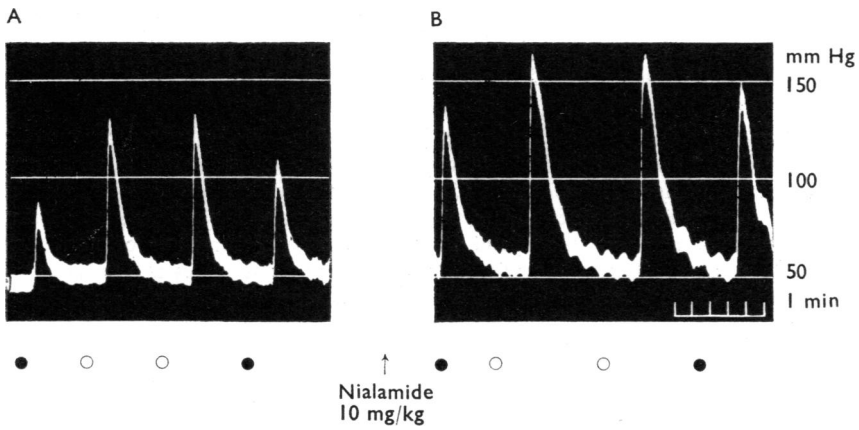


Fig. 2. Pithed cat. Blood pressure responses to intravenous injections of noradrenaline, after treatment with reserpine (5 mg/kg subcutaneously) 18 hr before acute experiment. Open circles: noradrenaline, 1 μg. Closed circles: noradrenaline, 0.5 μg. Between A and B (10 min), nialamide, 10 mg/kg intravenously.

of their limited uptake capacity, were saturated with noradrenaline, so that the amount of noradrenaline at the receptors remained unchanged regardless of any interference of uptake produced by nialamide.

The effect of nialamide on the action of tyramine in cats previously treated with reserpine

The effects of tyramine on the blood pressure and nictitating membrane of the cat are antagonized by prior treatment with reserpine (Carlsson, Rosengren, Bertler & Nilsson, 1957). These effects of tyramine can be restored by slow intravenous infusions of noradrenaline (Burn & Rand, 1958). In our experiments noradrenaline (20 μg/ml.) was infused at a rate of 2 ml./min for 10 min in order to restore temporarily the response to tyramine on both the blood pressure and the nictitating membrane in cats previously treated with reserpine (Fig. 3). This restoration of response by slow intravenous infusion did not last long; after three injections of tyramine at 10 min intervals the responses were at pre-infusion level. It was thus possible to evoke repeatedly a cycle of exhaustion of the effects of tyramine and their restoration by noradrenaline infusion. However, if nialamide (10 mg/kg) was injected during the cycle the responses were radically changed. The response of the blood pressure to tyramine was no longer restored by the infusion of noradrenaline, whereas the response of the nictitating membrane to tyramine steadily increased and became more and more prolonged (Fig. 3).

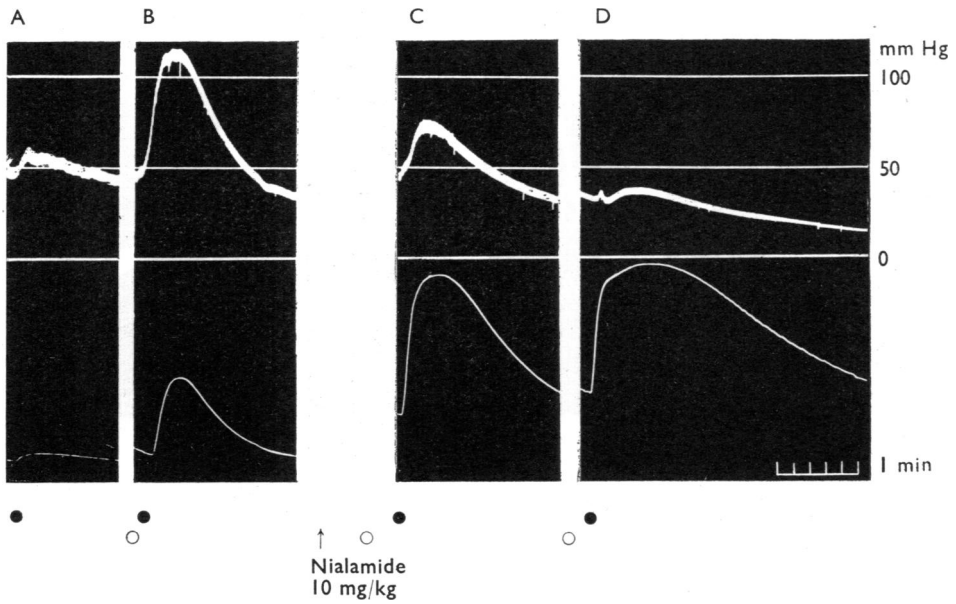


Fig. 3. Pithed cat. Upper tracing: arterial blood pressure. Lower tracing: contractions of nictitating membrane. Cat treated with reserpine (5 mg/kg subcutaneously) 18 hr before acute experiment. Closed circles: tyramine, 2 mg intravenously. Open circles: noradrenaline, 20 μg/ml. infused intravenously at a rate of 2 ml./min for 10 min. Between B and C, nialamide, 10 mg/kg intravenously.

After the infusion of noradrenaline, single injections of noradrenaline caused approximately 50% of their pressor response before infusion; this occurred both before and after the administration of nialamide, showing that this effect of nialamide was not the result of increased desensitization.

The action of nialamide on the effects of tyramine on the cat's blood pressure and nictitating membrane

The effects of repeated single injections of tyramine on the blood pressure and nictitating membrane were examined with three cats. Tyramine (2 mg) was injected at intervals of 15 min. In one cat there was no decline in the responses over a period of 180 min. In two cats there was a gradual decline in the effects of tyramine on the blood pressure and nictitating membrane (Fig. 4). Both these effects of tyramine were partially restored after an infusion of noradrenaline (40 μg/min for 10 min).

The action of nialamide on the effects of tyramine on the blood pressure and nictitating membrane was studied with three cats. A single intravenous injection of nialamide (10 mg/kg) was given after three injections of tyramine (2 mg) had been administered at regular intervals of 15 min. After nialamide the response of the blood pressure to tyramine was initially prolonged but then decreased rapidly. An infusion of noradrenaline failed to restore the effects of tyramine. With the nictitating membrane, however, after nialamide the response to tyramine steadily

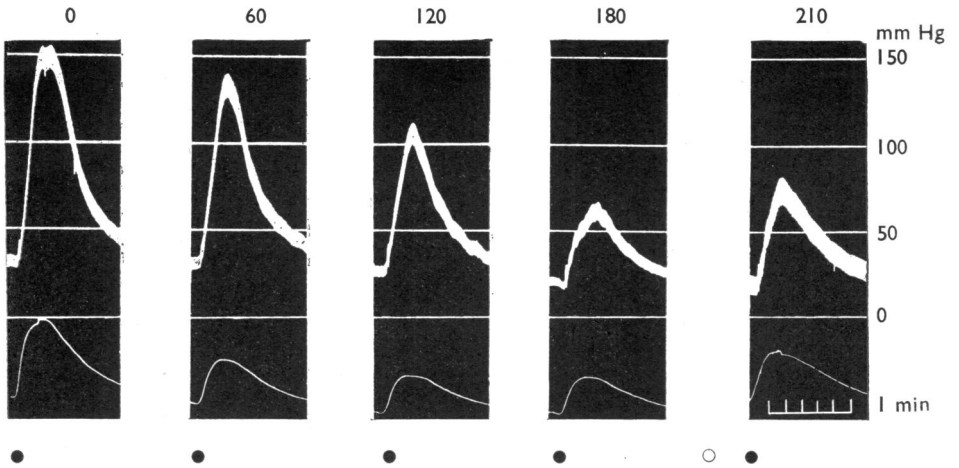


Fig. 4. Pithed cat. Upper tracing: arterial blood pressure. Lower tracing: contractions of nictitating membrane. Closed circles: tyramine, 2 mg intravenously. Open circle: noradrenaline, 20 μg/ml. infused intravenously at a rate of 2 ml./min for 10 min. Figures at top of tracing: time in min.

increased and became more and more prolonged, so that eventually the membrane was in a state of contracture (Fig. 5). When this contrast between the responses of blood pressure and the nictitating membrane to tyramine was at its peak, the blood pressure response to noradrenaline was normal, showing that the contrast was not due to some failure of arteriolar smooth muscle.

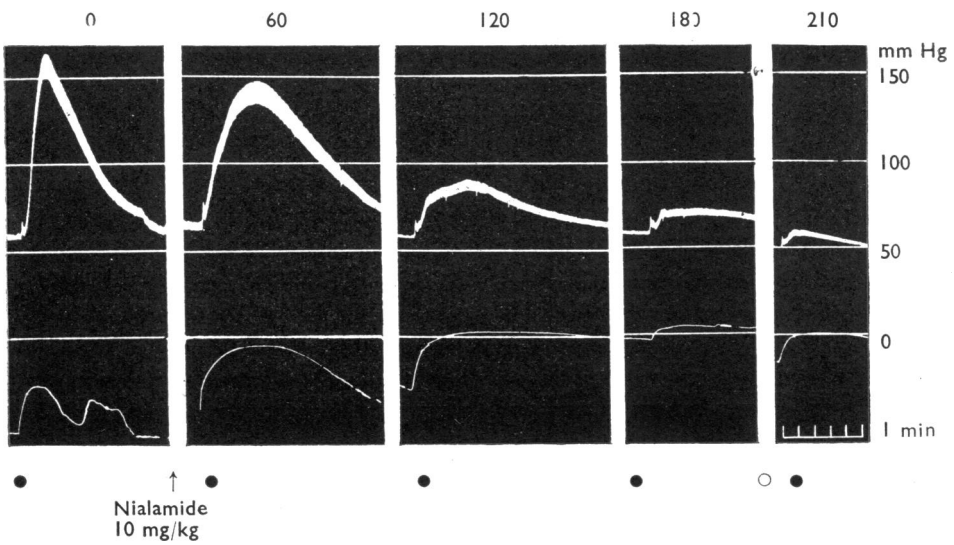


Fig. 5. Pithed cat. Upper tracing: arterial blood pressure. Lower tracing: contractions of nictitating membrane. Closed circles: tyramine, 2 mg intravenously. Open circle: noradrenaline, 20 μg/ml. infused intravenously at a rate of 2 ml./min for 10 min. At arrow, nialamide, 10 mg/kg intravenously. Figures at top of tracing: time in min.

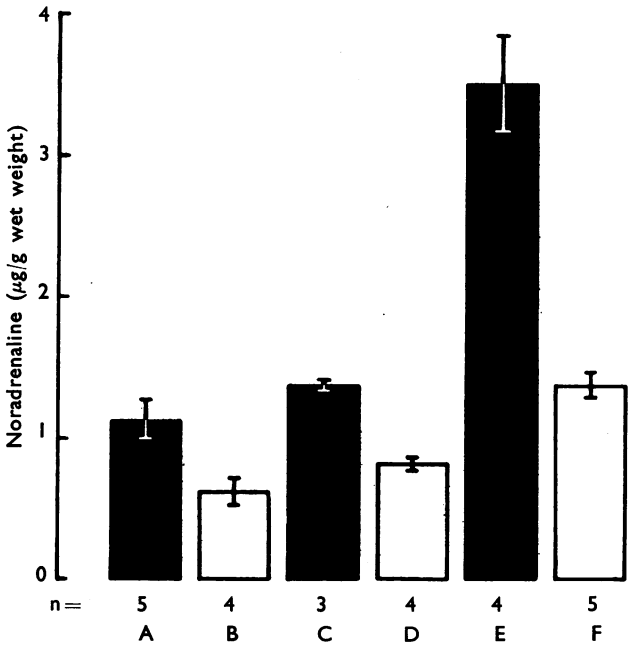


Fig. 6. The average myocardial concentrations of noradrenaline (ordinate, μg/g wet weight) in isolated perfused guinea-pig hearts. The vertical lines are the standard errors of the means. The figures below the columns give the number of experiments. A: hearts perfused with Krebs's solution for 180 min. B: hearts given tyramine (100 μg) at regular intervals of 5 min until tyramine no longer had a positive inotropic effect. C: hearts perfused with Krebs's solution containing nialamide (100 μg/ml., 200 ml./kg of body weight), followed by Krebs's solution for 60 min. D: hearts perfused with Krebs's solution containing nialamide (100 μg/ml., 200 ml./kg of body weight), followed by tyramine (100 μg) at regular 5 min intervals until tyramine no longer had a positive inotropic effect. E: hearts perfused with Krebs's solution for 60 min ; the guinea-pigs had received nialamide (20 mg/kg intraperitoneally) 72 hr before sacrifice. F: hearts given tyramine (100 μg) at regular 5 min intervals until tyramine no longer had a positive inotropic effect ; the guinea-pigs had received nialamide (20 mg/kg intraperitoneally) 72 hr before sacrifice.

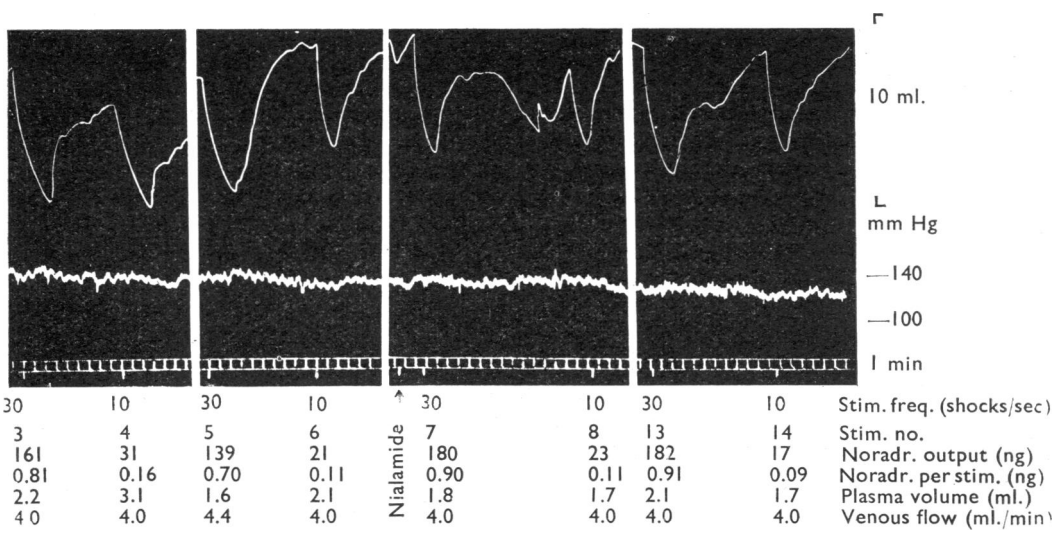
The effects of nialamide and tyramine on the concentration of noradrenaline in guinea-pig hearts

The isolated guinea-pig heart was used to test the hypothesis that nialamide interfered with the re-entry of noradrenaline into an endogenous storage site but not with noradrenaline release by tyramine. In order to determine whether the development of tachyphylaxis toward tyramine was associated with a reduction in the myocardial concentration of noradrenaline, guinea-pig hearts were repeatedly injected with 100 μg of tyramine at regular intervals of 5 min until tyramine no longer increased the amplitude of the contractions. The myocardial concentration of noradrenaline was then determined and expressed as μg/g wet weight. The total amount of tyramine administered before the disappearance of the inotropic cardiac response was from 2.1 to 3.4 mg in different experiments. When the inotropic effect of tyramine had disappeared, there was a reduction of approximately 40% in the

myocardial concentration of noradrenaline, compared with the concentration of noradrenaline in control hearts which had been perfused for 180 min without addition of tyramine (Fig. 6, A and B).

The effects of nialamide were determined either by including the drug in the Krebs's solution or by administration of a single intraperitoneal injection of nialamide to guinea-pigs 72 hr prior to sacrifice. In seven experiments, nialamide (100 μg/ml.) was added to the Krebs's solution, and hearts were perfused with this solution until the volume of perfusate was equivalent to 200 ml./kg of body weight. The dose was expressed in this way in order to compensate for variations in outflow rate and body weight. The perfusion was then continued with nialamide-free Krebs's solution. In four experiments tyramine (100 μg) was given at regular 5 min intervals until the drug no longer had a positive inotropic effect. In the remaining three experiments the perfusion was continued for 60 min. Nialamide affected neither heart rate nor force of myocardial contraction. The concentration of noradrenaline in hearts which had received tyramine was 0.97 μg/g (s.e. of mean, $\pm$0.054), and was 1.42 μg/g (s.e. of mean, $\pm$0.003) in hearts which had not received tyramine (Fig. 6, C and D). The total amount of tyramine administered before the positive inotropic effect of the drug disappeared ranged from 0.6 to 1.3 mg.

In nine experiments nialamide (20 mg/kg) was injected intraperitoneally 72 hr before the animals were sacrificed, and their hearts perfused. In five experiments tyramine (100 μg) was given at regular 5 min intervals until it no longer produced a positive inotropic effect. In four experiments the hearts were perfused for 60 min. The



30	10	30	10	30	10	30	10	Stim. freq. (shocks/sec)
3	4	5	6	7	8	13	14	Stim. no.
161	31	139	21	180	23	182	17	Noradr. output (ng)
0.81	0.16	0.70	0.11	0.90	0.11	0.91	0.09	Noradr. per stim. (ng)
2.2	3.1	1.6	2.1	1.8	1.7	2.1	1.7	Plasma volume (ml.)
4 0	4.0	4.4	4.0	4.0	4.0	4.0	4.0	Venous flow (ml./min)

Fig. 7. Effect of a close-arterial injection of nialamide (2 mg) into the isolated perfused cat's spleen. Spleen cat, 2.0 kg, male. Perfusion cat, 2.8 kg, male. Chloralose anaesthesia. Upper tracing: volume changes of the spleen, contractions downwards. Lower tracing: splenic perfusion pressure. At the signal marks the splenic nerves were stimulated at the frequencies indicated. Below these figures there is given in sequence: stimulus number; total output of noradrenaline (ng); output of noradrenaline per stimulus (ng); plasma volume of the venous outflow (ml.); total venous outflow (ml./min). At the arrow, nialamide, 2 mg, was injected close-arterially.

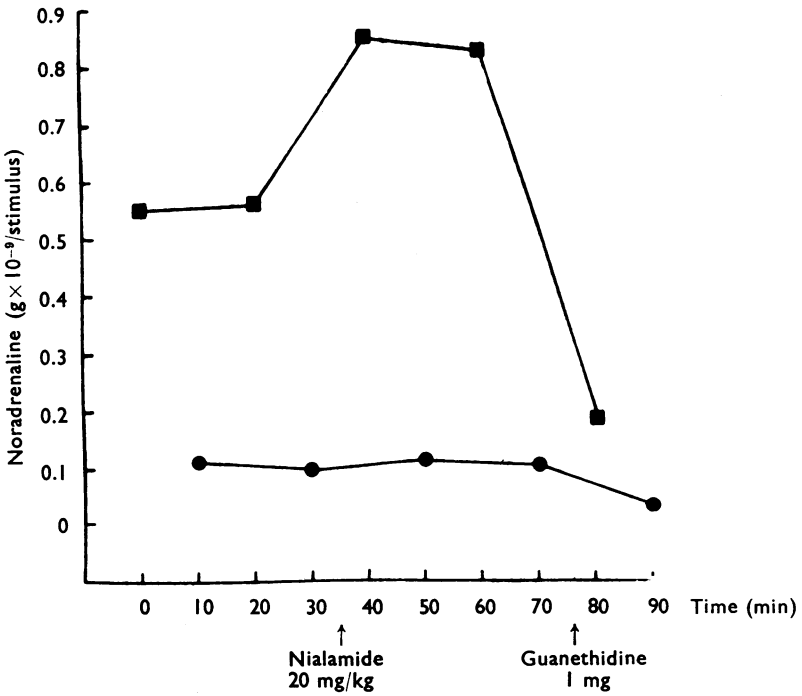


Fig. 8. Comparison of the effects of nialamide and guanethidine on the output of noradrenaline (ordinate) from the isolated perfused cat's spleen. Abscissa, time in min. ■—■—■ output of noradrenaline per stimulus at a stimulus frequency of 30 shocks/sec. ●—●—● output of noradrenaline per stimulus at a stimulus frequency of 10 shocks/sec. At the first arrow, nialamide, 20 mg/kg, was injected intravenously into the cat perfusing the spleen. At the second arrow, guanethidine, 1 mg, was injected close-arterially into the perfused spleen.

concentration of noradrenaline in the hearts which had received tyramine was 1.42 μg/g (s.e. of mean, ±0.102), and was 3.60 μg/g (s.e. of mean, ±0.35) in the hearts perfused with Krebs's solution only (Fig. 6, E and F). The total amount of tyramine administered before the inotropic effect of tyramine disappeared ranged from 0.4 to 1.5 mg.

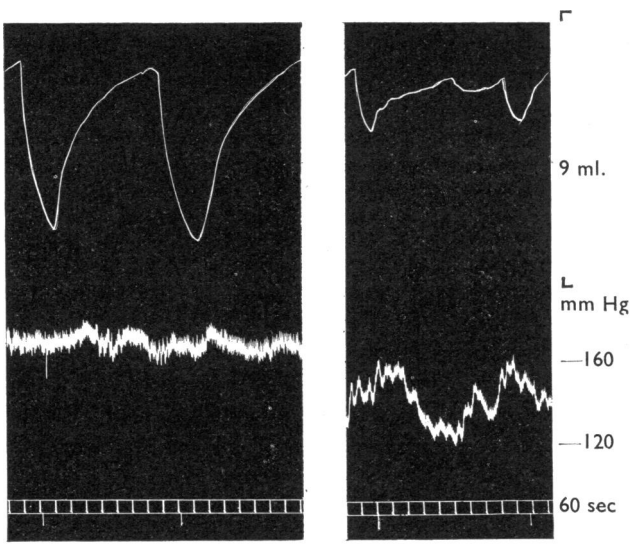
The acute effect of nialamide on the isolated cross-perfused cat spleen

In nine experiments nialamide, either 2 mg by close-arterial injection or 20 mg/kg of body weight intravenously, was given to a "donor" cat perfusing the spleen, and its effects on the splenic contractions and on the output of noradrenaline were studied. As Brown & Gillespie (1957) first described, the control output of noradrenaline per stimulus at a frequency of stimulation of 30 shocks/sec was about six times that at a frequency of 10 shocks/sec. Either of the two methods of administration of nialamide increased the output of noradrenaline at a stimulus frequency of 30 shocks/sec, but not at a frequency of 10 shocks/sec in five of the nine experiments (Figs. 7 and 8). In the remaining four experiments the output of noradrenaline was unchanged. Nialamide did not alter either the rate of blood outflow or the vasopressor activity of venous blood from the spleen in the absence

of splenic nerve stimulation. The spleen did not fail to contract in response to repeated periods of nerve stimulation; the first contraction after the close-arterial injection of nialamide was prolonged. In two experiments guanethidine (1 mg) was injected directly into the arterial supply to the spleen. The outputs of noradrenaline were decreased at both frequencies of stimulation (Fig. 8). Guanethidine (1 mg) caused a contraction of the capsular smooth muscle, but no significant increase in the vasopressor activity of the venous blood from the spleen could be demonstrated.

The chronic effects of nialamide on the isolated cross-perfused cat spleen

Nine experiments were carried out with spleens from cats to which nialamide (20 mg/kg) had been administered subcutaneously 20 hr before perfusion. In each experiment there was a progressive decline in the size of the contractions of the spleen in response to splenic nerve stimulation (Fig. 9). The outputs of noradrenaline per stimulus decreased at both frequencies of stimulation (Figs. 9 and 10). In one experiment the output of noradrenaline per stimulus at a frequency of stimulation of 30 shocks/sec was no greater than that at a frequency of 10 shocks/sec (Fig. 9).



10	30	10	30	Stim. freq. (shocks/sec)
1	2	11	12	Stim. no.
46.7	56.0	23.3	17.3	Noradr. output (ng)
0.23	0.28	0.11	0.08	Noradr. per stim. (ng)
3.5	2.8	1.4	1.3	Plasma volume (ml.)
4.5	4.5	4.0	4.0	Venous flow (ml./min)

Fig. 9. The effects of nialamide, 20 mg/kg subcutaneously, 20 hr before perfusion of the spleen. Spleen cat, 3.3 kg, male. Perfusion cat, 4.2 kg, male. Chloralose anaesthesia. Upper tracing: volume changes of the spleen, contraction downwards. Lower tracing: perfusion pressure. At the signal marks the splenic nerves were stimulated at the frequencies indicated. Below these figures are given in sequence: stimulus number; total output of noradrenaline (ng); output of noradrenaline per stimulus (ng); plasma volume of the venous outflow (ml.); total venous outflow (ml./min).

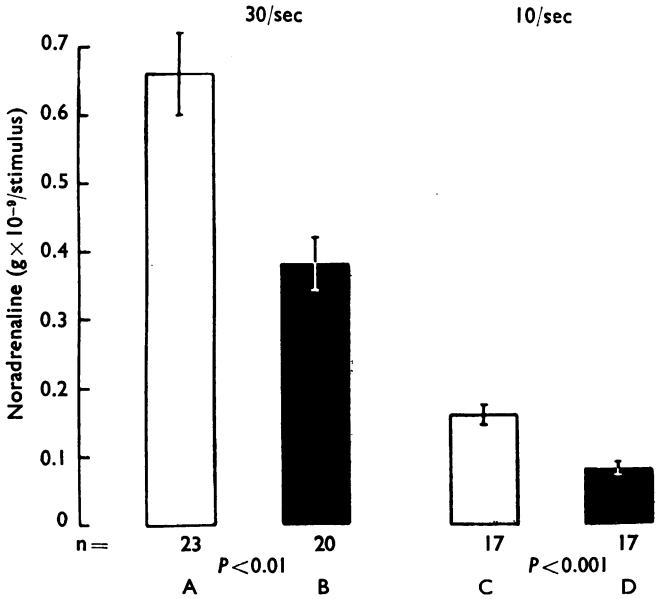


Fig. 10. Histogram of the average output of noradrenaline per stimulus at stimulus frequencies of 30 and 10 shocks/sec. The vertical lines are the standard errors of the mean. The columns represent the average outputs of noradrenaline per stimulus in the splenic venous blood. Open columns: control experiments. Solid columns: experiments on spleens from cats 18 hr after the subcutaneous injection of nialamide, 20 mg/kg. The figures immediately under the columns give the numbers of experiments.

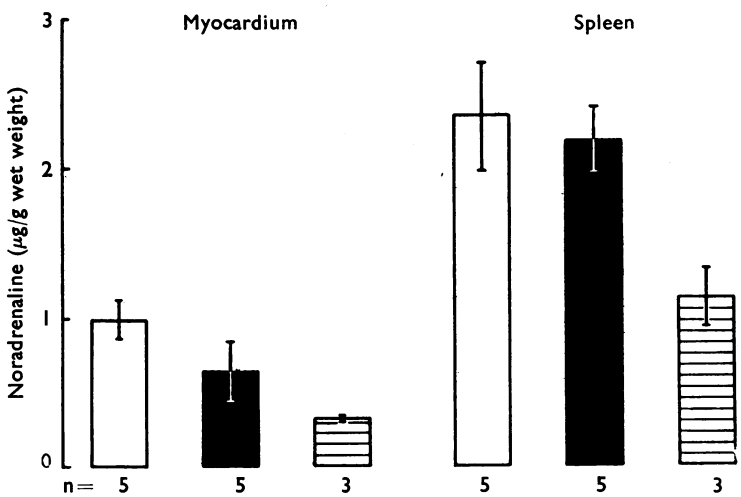


Fig. 11. Histogram of the average concentrations of noradrenaline (ordinate) in the myocardium and spleen of the cat. The vertical lines are the standard errors of the mean. Open columns: 18 hr after the cats had received 0.9% saline, 2 ml./kg, subcutaneously. Solid columns: 18 hr after nialamide, 20 mg/kg, subcutaneously. Hatched columns: after nialamide, 20 mg/kg subcutaneously, daily for 3 days. The figures below the columns give the numbers of experiments.

The effects of nialamide on the concentration of noradrenaline in the myocardium and spleen of the cat

Goldberg & Shideman (1962) reported that the myocardial concentrations of catechol amines were decreased by iproniazid and tranylcypromine in the cat but were increased in the rat. In the present series of experiments five cats received a single subcutaneous injection of nialamide (20 mg/kg) and five control animals received 2.0 ml./kg of 0.9% saline subcutaneously. After an interval of 20 hr anaesthesia was induced with ether and the cats made spinal. After 1 hr the heart and spleen were removed. Despite a trend in some experiments, there was under these conditions no significant decrease in the concentration of noradrenaline in either the spleen or the myocardium (Fig. 11). Thus no decrease in the tissue concentrations of noradrenaline could be demonstrated when nialamide had been previously administered in the same way as in the experiments in which the output of noradrenaline from the spleen was determined. However, the concentrations of noradrenaline in the myocardium and spleen were decreased, to 0.28 μg/g (s.e. of mean, $\pm$0.027) and 1.14 μg/g (s.e. of mean, $\pm$0.324) respectively, in three cats which received nialamide (20 mg/kg) subcutaneously on each of the three consecutive days preceding sacrifice (Fig. 11).

DISCUSSION

Immediately after the intravenous injection of nialamide the pressor effects of tyramine could no longer be restored by slow intravenous infusions of noradrenaline in cats with their tissue stores of noradrenaline depleted by prior treatment with reserpine. Only in cats previously treated with reserpine did nialamide immediately potentiate the pressor effects of noradrenaline. One explanation of these two observations is that nialamide interfered with the penetration of noradrenaline into a storage site of rather limited capacity for uptake. The restricted capacity for uptake of this site was suggested by the observation that nialamide did not potentiate the pressor effects of noradrenaline in cats which had not previously been treated with reserpine, i.e., in cats in which the binding sites were saturated with endogenous noradrenaline. Nialamide gradually decreased the pressor responses to repeated injections of tyramine in pithed cats, in contrast to guanethidine which immediately antagonizes the pressor effects of tyramine. The fact that nialamide immediately antagonized the restorative effect of noradrenaline and gradually decreased the pressor effects of repeated injections of tyramine suggests that nialamide interferes with the re-entry of noradrenaline but not with its release by tyramine.

The experiments with isolated guinea-pig hearts support this hypothesis. The development of tachyphylaxis to tyramine in the isolated guinea-pig heart was associated with a 40% reduction in the myocardial concentration of noradrenaline. This observation is not compatible with the results of Nasmyth (1960), who considered that the loss of the inotropic response to tyramine in the isolated guinea-pig heart was not related to the intracellular concentration of noradrenaline. In the present series of experiments the concentration of noradrenaline in the myocardium was determined, whereas Nasmyth estimated the cardiac " pressor amine " content which, since the endogenous stores of catechol amines seem to act like ion-exchange resins, may well have been a mixture of noradrenaline and

tyramine. The onset of tachyphylaxis towards the inotropic effect of tyramine was much more rapid when nialamide was either included in the perfusion fluid or administered to the guinea-pigs prior to sacrifice. The decrease in the myocardial concentration of noradrenaline was not prevented. This effect could also be explained by the hypothesis that nialamide interfered with the re-entry of noradrenaline but not with its release by tyramine. The tachyphylaxis towards the positive inotropic effect of tyramine in hearts taken from guinea-pigs treated with nialamide occurred when the myocardial concentration of noradrenaline had decreased from 3.60 to 1.42 μg/g, whereas the development of tachyphylaxis in normal hearts occurred when the myocardial concentration of noradrenaline had fallen from 1.15 to 0.67 μg/g. This suggested that tyramine could only release a certain proportion of the noradrenaline present in the heart, and that the absolute amount of noradrenaline present was not the factor which determined the presence or absence of a response to tyramine.

The marked difference in the behaviour of the smooth muscle of the nictitating membrane compared with that of the vascular system may be due to a difference in the relative importance of the enzyme monoamine oxidase in the inactivation of tyramine in these two situations in the cat.

Hertting & Axelrod (1961), using [^{3}H]-noradrenaline, have shown that a certain proportion of released noradrenaline re-enters the nerve endings, thus producing an economy of transmitter, as proposed by Marley & Paton (1961). In the present work the effects of nialamide on the output of noradrenaline from the spleen were studied to explore the possibility that nialamide inhibited this re-entry of noradrenaline and thus eventually decreased the availability of transmitter.

The acute administration of nialamide increased the output of noradrenaline per stimulus when the stimulus frequency was 30 shocks/sec, but not with 10 shocks/sec (five of nine experiments). This could indicate that less noradrenaline re-entered the nerves, for Brown & Gillespie (1957) showed that when the tissue receptors for noradrenaline were blocked with either dibenamine or dibenyline then the output of noradrenaline per stimulus at a stimulus frequency of 10 shocks/sec became equal to that at 30 shocks/sec. It may be that nialamide did not increase the output of noradrenaline at a stimulus frequency of 30 shocks/sec in four experiments because the " re-entry sites " were sufficiently saturated with noradrenaline. The decreased output of noradrenaline per stimulus from spleens taken from animals which had been treated with nialamide suggests that nialamide, as a result of its inhibition of the re-entry of noradrenaline, eventually decreased the availability of transmitter. This concept is supported by the observation that nialamide, which does not release catechol amines, decreased the concentrations of noradrenaline in the myocardium and spleen of the cat.

Nialamide only potentiated the pressor effects of noradrenaline in cats in which the tissue stores of catechol amines had been depleted, which suggests that the storage site involved is of rather limited capacity for uptake. The existence of such a site is indicated by the fact that the effects of sympathetic nerve stimulation in animals treated with reserpine can be restored by noradrenaline and its precursors (Burn & Rand, 1960 ; Gillespie & Mackenna, 1961), whereas Muscholl (1960) could

not demonstrate any uptake of noradrenaline into the heart of a rat previously treated with reserpine.

The experimental results support the hypothesis that nialamide diminishes the availability of transmitter by decreasing re-entry of noradrenaline into a storage site in nerve endings which is of rather limited capacity for uptake. A decreased availability of transmitter in hyperactive nerve pathways may account for the antihypertensive effects of monoamine oxidase inhibitors in man.

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