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Effects of Dibenamine on Cardiovascular Actions of Epinephrine, Acetylcholine, Pitressin and Angiotonin in Unanesthetized Dogs.*

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Nickerson and Goodman[1] have described adrenolytic actions of N, N-dibenzyl beta-chloroethyl amine (Dibenamine). They found that the compound, when injected slowly intravenously, produced minimal effects upon arterial blood pressure and, within 30 minutes after the injection, the pressor action of epinephrine was blocked in some species and reversed in others. The effects of stimulating various excitatory adrenergic nerves and the excitatory actions of epinephrine upon various effectors were prevented by Dibenamine. On the other hand, the inhibitory actions of epinephrine upon the non-pregnant cat uterus *in situ* and on the isolated small intestine of the rabbit and rat were not prevented by Dibenamine. They also observed that larger doses were required to block the effects of stimulating excitatory adrenergic nerves than were required to block the excitatory effects of injected epinephrine. Cardio-accelerator actions of epinephrine were not blocked by Dibenamine.

The present study is concerned principally with the cardiovascular actions of Dibenamine and with its effects upon the cardiovascular responses to epinephrine, acetylcholine, pitressin, and angiotonin in unanesthetized dogs.

Methods. Unanesthetized animals were trained to lie quietly on the table while a needle was kept in place in the radial vein. Heart rate was determined by counting the apex beat and from continuous electrocardiographic records taken during injection of the 4 test compounds. In one animal the spinal cord had been transected 24 hours previously and blood pressure was recorded from the femoral artery by the use of a mercury manometer.

N, N-dibenzyl beta-chloroethyl-amine hydro-chloride was prepared for injection by dissolving 200 mg in approximately 15 cc of propylene glycol, and this was diluted with an equal amount of water. Injection of the solution was started within 2 to 5 minutes after preparation, and a period of 5 to 20 minutes was required to give the animal the total dose of 200 mg.

Seven dogs weighing between 9 and 13 kg were used. Six of these were intact, and one had the spinal cord transected. The effects of test doses of epinephrine, acetylcholine, pitressin, and angiotonin upon heart rate were recorded before and at stated intervals after administration of Dibenamine. During each series of injections a needle was placed in the radial vein and kept open by injecting small amounts of isotonic saline. At the desired time a syringe containing the exact amount of the compound to be administered was substituted for the syringe containing saline and the compound was injected rapidly. Ample time was allowed for return of the heart rate to the resting level between injections.

Results. A. *Effects of Dibenamine on heart rate.* Dibenamine alone caused an increase in heart rate in each of the 6 intact animals. The maximal increase above the basal rate ranged from 46% to 95%, and it occurred in 10 to 25 minutes from the beginning of the injection. Usually the rates returned to the resting level within one to 3 hours.

B. *Effects of epinephrine on heart rate before and after Dibenamine.* Continuous electrocardiographic records were obtained during the test dose of epinephrine (2 cc of a 1-100,000 dilution) in 4 dogs before and after administration of Dibenamine. The averages of the rates for the 4 animals are graphed in Fig. 1.

The test dose of epinephrine produced a

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1 Nickerson, M., and Goodman, L., *J. Pharm. and Exp. Therap.*, 1947, **89**, 167.

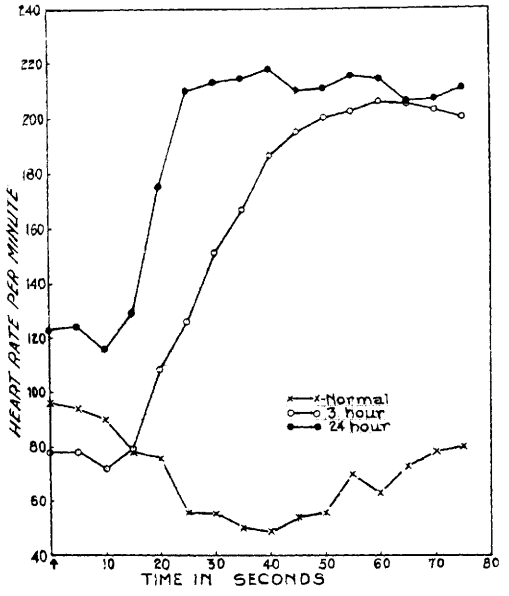

FIG. 1.

maximal decrease in rate of 45% to 57% in the 4 intact animals before Dibenamine. Since the direct influence of epinephrine upon the sino-auricular node is excitatory, this bradycardia is best explained on the basis of reflex effects elicited by a rise in blood pressure.

Three hours after Dibenamine had been administered the average heart rate was 19 beats per minute slower than the average of the control rates. At this time the test dose of epinephrine produced an acceleration of 97% to 208% above the rate preceding the injection. Acceleration was clearly evident within 20 seconds after the injection of epinephrine and progressed smoothly to a plateau at about 50 seconds. It was still maintained at 75 seconds. The effects of epinephrine on the heart rate 24 hours after Dibenamine were similar to the effects seen 3 hours after Dibenamine.

The reversal of the effect of the test dose of epinephrine on the heart rate of unanesthetized animals by Dibenamine may be readily explained if it is considered that epinephrine causes a fall in blood pressure in the unanesthetized animals after they have received Dibenamine. If such is the case, a smooth cardiac acceleration would be expected because of the combination of the direct accelerator influence of epinephrine and the reflex accelerator influence of the fall in blood pressure. This interpretation is supported by the observation that in an unanesthetized animal, with spinal cord transected at T_{12} so that blood pressure could be recorded from the femoral artery, injection of the test dose of epinephrine, after the animal had been given Dibenamine, caused an immediate fall of 50 mm of mercury in the mean arterial blood pressure. Animals under the influence of Dibenamine also characteristically showed much greater respiratory stimulation after the epinephrine injection than was seen in the unmedicated animals. Presumably, this respiratory stimulation is elicited reflexly from the fall in blood pressure.

The striking cardiac acceleration in response to epinephrine after Dibenamine would seem to indicate that the cardiac effects of epinephrine or of cardio-accelerator nerves are not blocked. However, a large part of this acceleration could be on the basis of decreased tonus of cholinergic cardio-inhibitory nerves.

C. *Effects of Dibenamine on the cardio-accelerator response to acetylcholine.* The brief hypotension produced by intravenous injection of acetylcholine elicits a typical cardio-accelerator response. The acceleration is due largely to reflex activation of adrenergic nerves and to liberation of epinephrine

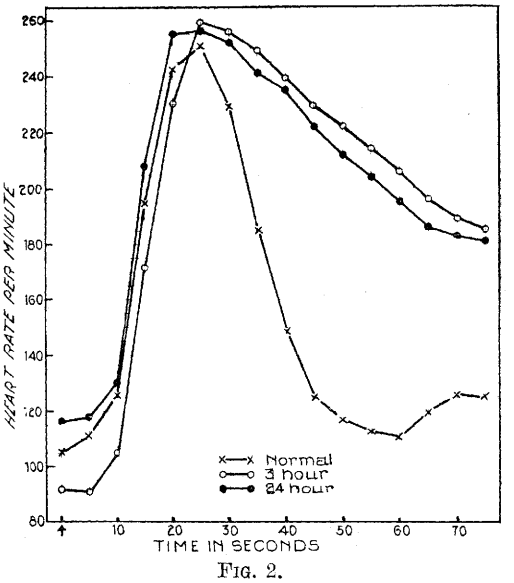

FIG. 2.

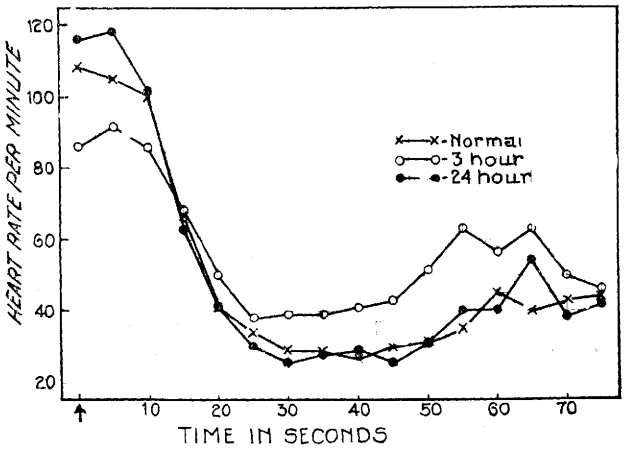

FIG. 3.

from the adrenal medulla.[2] Five animals were given a test dose of 1 mg of acetylcholine at 3 hours and again at 24 hours after the administration of Dibenamine. In Fig. 2 a curve is shown which illustrates the average of the cardio-accelerator responses to the test dose of acetylcholine in 23 animals. The other 2 curves in Fig. 2 illustrate the averages of the cardio-accelerator response to acetylcholine in 4 animals at 3 hours and at 24 hours after Dibenamine. The degree of acceleration was at least as great after Dibenamine as before. In both the normal animals and in those under the influence of Dibenamine, the maximum increase in heart rate in response to acetylcholine occurred at 25 seconds. In the normal animals the rate fell off rapidly, to reach a resting level within 50 to 60 seconds, but in the animals under Dibenamine the return to the resting level was quite delayed. The persistence of the fast rate would indicate that Dibenamine interferes with the restoration of the blood pressure to the normal level after a test dose of acetylcholine. This result is compatible with the interpretation that vasoconstrictor mechanisms are impaired by Dibenamine while cardio-accelerator mechanisms are relatively intact.

D. *Effects of Dibenamine upon the cardio-inhibitory response to pitressin and angiotonin.* The effects of Dibenamine on the pressor actions of pitressin and angiotonin were tested in 3 dogs which had received sodium pentobarbital. At the time when the pressor action of epinephrine was reversed, there was no interference with the pressor actions of either pitressin or angiotonin. In one animal under sodium pentobarbital, neurogenic hypertension was produced by sino-aortic denervation. When Dibenamine was injected in this animal, it produced a profound fall in blood pressure.

In unanesthetized dogs pitressin and angiotonin produce a pronounced reflex bradycardia during the rise in blood pressure resulting from their vasoconstrictor action.[3] If the vasoconstrictor action of these compounds is not blocked by Dibenamine, they would be expected to produce the typical cardio-inhibitory response. The effects of a test dose of 1½ pressor units of pitressin before and after Dibenamine were studied in 4 dogs, and the effects of a test dose of 20 pressor units of angiotonin were studied in 3 dogs. The averages of the cardio-inhibitory responses to pitressin in 4 dogs at 3 hours and at 24 hours after Dibenamine are shown in Fig. 3, and these are compared with a curve showing the averages of 14 normals. The results of a typical experiment with angiotonin are graphed in Fig. 4.

From these results it is evident that Dibena-

[2] Youmans, W. B., Aumann, K., Haney, H., and Wynia, F., *Am. J. Physiol.*, 1940, **128**, 467.

[3] Haney, H. F., Lindgren, A. J., Karstens, A. I., and Youmans, W. B., *Am. J. Physiol.*, 1943, **139**, 675.

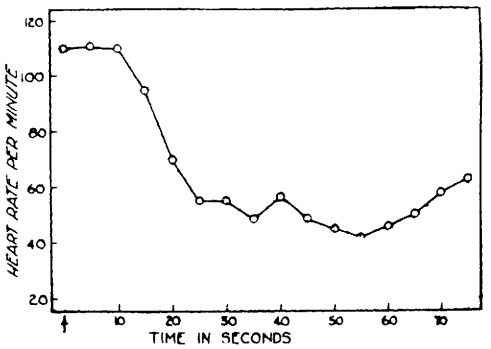

FIG. 4.

mine does not alter the cardio-inhibitory responses produced by the injection of pitressin and angiotonin. This would indicate that, as in the anesthetized animals, Dibenamine does not impair the vasoconstrictor actions of these compounds.

Summary and Conclusions. The actions of Dibenamine on the cardiovascular adjustments caused by epinephrine, acetylcholine, pitressin, and angiotonin have been studied in unanesthetized dogs. Dibenamine produced an increase in heart rate which persisted for one to 3 hours.

A test dose of epinephrine which regularly caused reflex cardiac slowing in normal unanesthetized dogs produced severe cardiac acceleration after administration of Dibenamine. This acceleration is attributable to the direct stimulatory action of epinephrine on the sino-auricular node and to reflex acceleration from a fall in blood pressure.

The compensatory cardiac acceleration produced by injection of a test dose of acetylcholine was undiminished and prolonged in unanesthetized animals under the influence of Dibenamine. This result is compatible with the interpretation that the vasoconstrictor mechanisms are impaired while the cardioaccelerator mechanisms are relatively intact.

The severe cardiac inhibition produced by pitressin and angiotonin in unanesthetized dogs, which is attributable to reflexes elicited by the rise in arterial blood pressure subsequent to vasoconstriction, was not prevented by Dibenamine.

The results of these experiments on unanesthetized dogs are in accord with the interpretations of Nickerson and Goodman[1] concerning the sites of action of Dibenamine.

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Diabetogenic Effect of Two Synthetic Estrogens in Force-Fed, Alloxan-Diabetic Rats.

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Ingle[1] has reported on the diabetogenic effect of diethylstilbestrol, dihydrostilbestrol, estradiol and equilin in the force-fed, partially depancreatized rat. More recently it was shown by Ingle, Nezamis and Prestrud[2] that diethylstilbestrol will intensify the glycosuria of rats having alloxan diabetes when the food intake is kept constant by forced feeding. The present study was a partial test of the hypothesis that compounds which are estrogenic are also diabetogenic in the rat. Substance I (2,4-di[p-hydroxyphenyl]-3-ethyl hexane) was described by Blanchard, Stuart and Tallman[3] and Substance II (1,2-dimethyl-2-carboxy-7-methoxy - 1,2,3,4,9,10-hexahydrophenathrene) was described by Hogg.[4]

Each of these two compounds belongs to a chemically different series of synthetic estrogens than any of the substances which have previously been examined for diabetogenic

1 Ingle, D. J., *Endocrinology*, 1941, **29**, 838.

2 Ingle, D. J., Nezamis, J. E., and Prestrud, M. C., *Endocrinology*, 1947, **41**, 207.

3 Blanchard, E. W., Stuart, A. H., and Tallman, R. C., *Endocrinology*, 1943, **32**, 307.

4 Hogg, J. A., *J. Am. Chem. Soc.*, in press.