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FURTHER STUDIES ON SYMPATHIN

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In recent years it has been shown that a substance with the physiological properties of synthetic *l-nor*adrenaline occurs as a regular constituent of adrenergic nerves (Bacq and Fischer, 1947; Tainter, Tullar, and Luduena, 1948; Euler, 1948; Graham, 1949), and probably plays an important role as a chemical nerve transmitter (Cannon and Rosenblueth, 1933). It occurs in various organs, including the adrenal medulla (Schumann, 1948; Holtz and Schumann, 1949; Bülbring and Burn, 1949a; Euler and Hamberg, 1949), and in medullary tumours (Holton, 1949; Goldenberg, Faber, Alston, and Chargaff, 1949). In addition, it has been isolated and identified from natural sources such as commercial adrenaline preparations (Tullar, 1949) and cattle adrenals (Bergstrom, Euler, and Hamberg, 1949).

Bülbring and Burn (1949b) showed that splanchnic nerve stimulation (after evisceration and ligation of the renal vessels in spinal cats) resulted in the liberation of varying proportions of *nor*adrenaline with adrenaline. Their results were obtained in single animals by comparing the height of contraction of the denervated nictitating membrane with that of the normal membrane. They found that relatively more *nor*adrenaline than adrenaline was produced on successive stimulation, but in cats fed with methionine the gradual decline in the proportion of adrenaline secreted during repeated splanchnic stimulation seemed to be absent; this suggested that the gland was capable of methylating *nor*adrenaline in order to increase its store of adrenaline, proof of which has now been found by Bülbring (1949), using suspensions of ground dogs' and cats' suprarenals, and by Bülbring and Burn (1949c) in perfused dog suprarenal gland experiments. We have confirmed the result that *nor*-adrenaline is liberated with adrenaline when the splanchnic nerve is stimulated by injecting the blood from a cat's suprarenal vein, after splanchnic stimulation, into the arteries supplying the chronically denervated nictitating membrane and acutely denervated non-pregnant uterus of a second cat, and comparing the effects with those produced by intra-arterial doses of adrenaline or *nor*adrenaline.

The theory that the substance liberated on stimulation of the hepatic nerves in the cat is *nor*adrenaline or some similar substance was put forward by Bacq (1934), Stehle and Ellsworth (1937), and Greer, Pinkston, Baxter, and Brannon (1937, 1938). Gaddum and Goodwin (1947) found no evidence against this theory that liver sympathin is *nor*adrenaline. Further similarity in the effects of hepatic nerve stimulation and intraportal injections of *nor*adrenaline was recorded by West (1948). Guanidine or cocaine, for example, in suitable doses did not potentiate the action of liver sympathin or of intraportal doses of *nor*adrenaline, whereas the pressor action of intraportal adrenaline was readily increased.

Stimulation of the splenic nerve in cats was found by West (1948) to produce a pure rise in blood pressure (partly due to the increase in circulating blood volume), which was not potentiated by intra-arterial injections of cocaine or guanidine. Intra-arterial doses of *nor*adrenaline produced a pure rise in blood pressure whereas doses of adrenaline produced a biphasic response, a large fall in blood pressure following the initial small rise. In a study of the nature of splenic sympathin, Peart (1949) tested blood from the splenic vein after stimulation on several isolated tissues, and all the evidence supported the view that the substance was *nor*adrenaline in a concentration of 50 to 500 mμg./ml. By a procedure similar to that described under the splanchnic nerve stimulation, we have been able to show that hepatic and splenic sympathin appears to consist almost entirely of *nor*adrenaline.

Injections of progesterone reverse from relaxation to contraction the responses of the non-pregnant uterus of the cat to stimulation of the hypogastric nerve or to injections of adrenaline. Kennard (1937) found that the reversal of responses to nerve stimulation in ovariectomized cats precedes that of responses to adrenaline. For a period of time the non-pregnant uterus under progesterone treatment is contracted by nerve stimulation and relaxed by adrenaline. Later, Labate (1941) showed that cocaine potentiated the actions of adrenaline and nerve stimulation on the uterus of non-pregnant cats, but in pregnant animals slight differences were noted. He considered that the important factor for the reversal was the oestrogen–progesterone antagonism. Bacq and Fischer (1947) suggested that the mediator in the cat uterus might be *nor*adrenaline and not adrenaline. It seemed probable from earlier work (Mann, 1949) that uterine sympathin in the rat might be *nor*adrenaline and not adrenaline, but by the techniques described it has not been possible to reach any conclusion in the cat, probably owing to the small quantities of sympathin liberated.

The present paper describes attempts to identify sympathin derived from four sources in the cat.

METHODS

Cats anaesthetized with chloralose were used in all experiments. Blood pressure records were taken from the right carotid artery, and injections of the drugs or of blood samples were made into the arteries supplying the nictitating membrane (right carotid) and the non-pregnant uterus (left external iliac), or into the femoral or splenic veins. Contractions of the nictitating membrane were recorded isotonically, usually 7–10 days after denervation by removal of the superior cervical ganglion. The uterus was fixed at its lower end after acute denervation and its movements were recorded directly. For experiments with single cats, the splanchnic, hepatic, splenic, or hypogastric nerves were separated and divided centrally. The *in vivo* work on uterine sympathin was completed in the morning and afternoon and then the uteri were removed for the *in vitro* experiments in the evening. The uteri were suspended in 30 ml. Tyrode solution at 37° C.

For later work, a second cat served to supply the blood samples. This animal was eviscerated for the experiments with adrenal sympathin, and its left splanchnic nerve separated and divided centrally. The left suprarenal vein was cannulated and when not required for injection into the recipient cat the blood was returned to the donor cat via the femoral vein. For the experiments with hepatic sympathin, the hepatic nerve was separated and divided centrally. Blood samples were taken from the hepatic vein by means of a syringe and a No. 15 needle (D'Silva, 1936). In other experiments, the splenic nerves were dissected free from the artery and divided

centrally, vascular connexions of the spleen with the stomach and greater omentum being divided between ligatures. The combined splenic vein was cannulated, and, when not required, the blood was returned to the donor cat via the femoral vein.

Stimulation of the nerves was through platinum electrodes with an ordinary coil or a square-wave stimulator (providing impulses of 2 volt intensity, 0.1 millisec. duration and on 22–50 cycles per sec.). In most experiments, cocaine hydrochloride (8 mg./kg.) was given intramuscularly. Dibenamine (15 mg./kg. intravenously) was used as the sympatholytic agent. Solutions of *l*-adrenaline and *l-nor*adrenaline were prepared with 0.01 N-HCl.

RESULTS

Adrenal sympathin

In single cats under chloralose and cocaine and after double vagotomy, the results of splanchnic nerve stimulation corresponded to those produced by intravenous infusions of adrenaline, since equiactive effects on the non-pregnant uterus, blood pressure, and nictitating membrane were obtained. If, however, the nerve was cut and left for several hours, weak stimulation then resulted in actions on the blood pressure and nictitating membrane which were equated by slow intravenous infusions of a mixture of *nor*adrenaline and adrenaline or sometimes of *nor*adrenaline only; little action on the non-pregnant uterus was obtained. This result suggested that the nature of adrenal sympathin depends in part upon the length of time the nerve has been sectioned before being stimulated, methylation of the primary amine possibly being reduced (West, 1949). On the other hand, Bülbring (1949) showed that methylation is much more active in glands which have been stimulated through the splanchnic nerve just before they were minced than in non-stimulated glands removed as quickly as possible under the most favourable conditions.

In an effort to obtain a quantitative estimate of the relative amounts of each amine, it was decided to inject the blood immediately after withdrawal from a stimulated eviscerated cat into the arteries supplying the nictitating membrane and non-pregnant uterus of a second cat under chloralose and cocaine. Blood from the suprarenal vein before stimulation showed no action on the blood pressure and uterus and only a trace on the membrane. Immediately after sectioning and stimulating the splanchnic nerve, the blood in many cases produced results corresponding to injections of adrenaline by both routes. With continued stimulation, the action on the uterus slowly disappeared, and by injecting mixtures of the two amines it was possible to show that the percentage of adrenaline in the sympathin present in the suprarenal blood usually decreased (Table I). In one cat (No. 8) only 20 per

TABLE I

Time in hours	Percentage of adrenaline released by the adrenal gland at various times by continuous stimulation of the splanchnic nerve in cats, Nos.:									
	1	2	3	4	5	6	7	8	9	10
0	100	100	100	100	100	100	80	20	100	80
0.5	—	—	—	—	—	75	—	0	100	80
1.0	50	50	—	—	50	20	20	—	70	—
1.5	—	—	20	—	—	—	—	—	30	—
2.0	—	—	—	20	—	—	—	—	—	80

cent adrenaline could be accounted for at the commencement of the experiment and this was reduced after half an hour (Fig. 1), whilst in another (No. 10) after two hours' continuous stimulation the adrenaline output had not decreased. In nearly all the

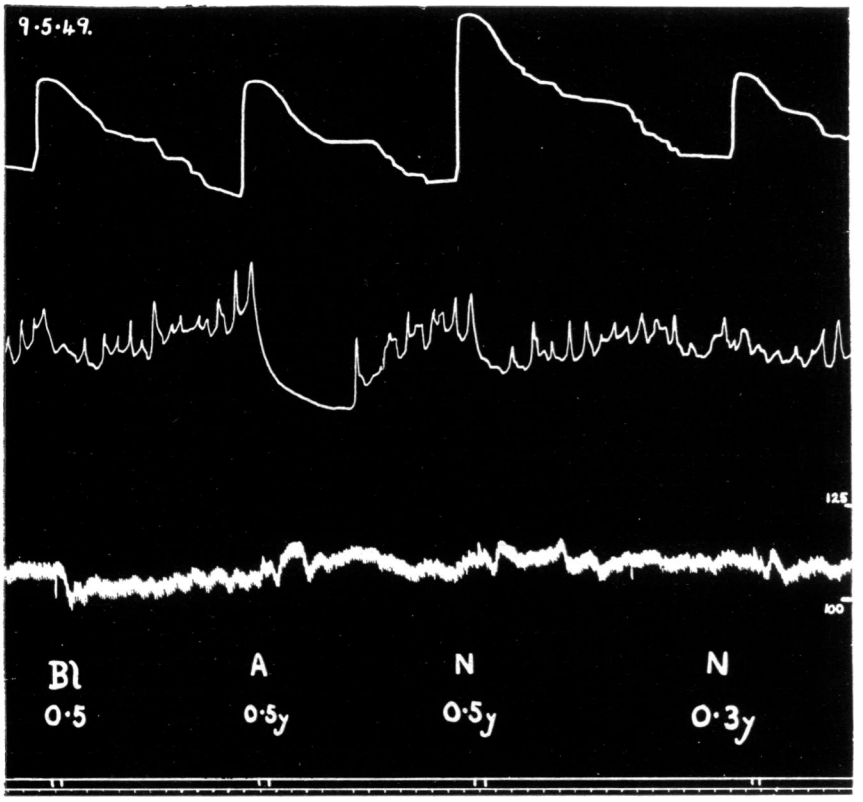

FIG. 1.—Cat, 3 kg. Chloralose. Cocaine. Record from top downwards: denervated nictitating membrane, non-pregnant uterus, blood pressure. Injections given into (1) external iliac artery, and (2) carotid artery; Bl 0.5 = 0.5 ml. blood from suprarenal vein of another cat after stimulation of the left splanchnic nerve. The result can be matched by a dose of *nor*adrenaline (N) and not by adrenaline (A).

other experiments where a large reduction of adrenaline output occurred, an increase in the strength of the stimulus (from 2 volts to 4 volts) resulted in a temporary rise in adrenaline output. Gaddum and Lembeck (1949) have recently confirmed that *nor*-adrenaline is present by carrying out parallel quantitative assays on the rat's uterus and colon.

In two other cats, 6 mg. potassium chloride was injected into the central end of the cut coeliac artery of the donor cat in order to stimulate the adrenal medulla; with this procedure, blood from the suprarenal vein was found to contain a mixture of approximately equal parts of adrenaline and *nor*adrenaline (not adrenaline only as Feldberg and Guimarais (1936) had suggested).

Liver sympathin

In single cats, stimulation of the hepatic nerves immediately after sectioning almost invariably caused a rise of blood pressure, part of which was due to constriction of the hepatic artery. Contraction of the nictitating membrane and relaxation of the non-pregnant uterus were also observed, these three actions being equated by slow intraportal infusions of a mixture of *nor*adrenaline and adrenaline. Later, stimulation produced a similar rise of blood pressure and reduced contraction of the nictitating membrane but had little or no action on the uterus, effects equated by slow intraportal infusions of equipressor doses of *nor*adrenaline.

When the blood from the hepatic vein was collected in a syringe (D'Silva, 1936) during stimulation of the hepatic nerve, and injected into the arteries supplying the nictitating membrane and uterus of another cat, the results corresponded to those produced by intra-arterial injections of *nor*adrenaline either alone or with a trace of adrenaline. Later estimates on the nictitating membrane and uterus indicated that *nor*adrenaline only was present in a concentration of 0.4 μg. per ml. blood.

Further similarity in the effects of hepatic nerve stimulation and intraportal injections of *nor*adrenaline is shown by using 2-benzyl-1-methyl-imidazoline. Gowdey (1948) reported that this drug potentiated the pressor action of adrenaline when injected into the splenic vein so as to pass through the portal system before entering the general circulation. In five cats, when this experiment was repeated with *nor*adrenaline, no such potentiation was noted (Fig. 2). Very slight potentiation of the action of liver sympathin occurred but this was quickly eliminated.

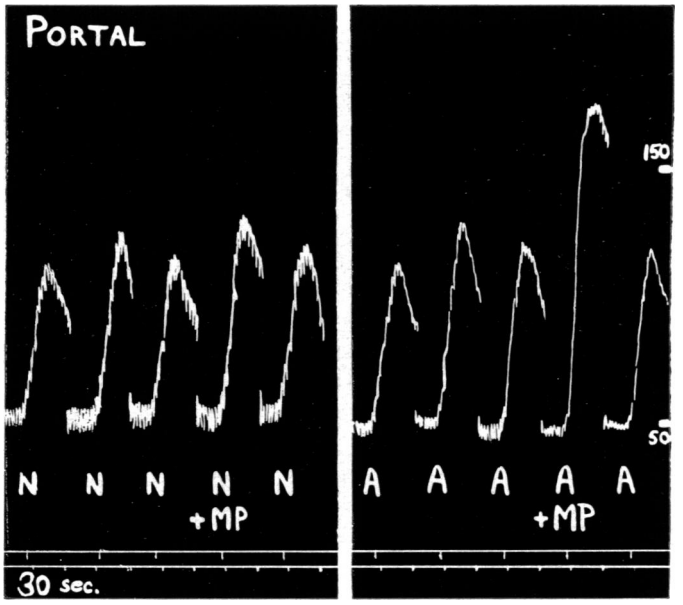

FIG. 2.—Spinal cat, 2 kg. Blood pressure record. All injections into the portal circulation: 2 mg. 2-benzyl-1-methyl-imidazoline (M.P.) greatly increases the pressor action of 20 μg. adrenaline (A) but scarcely affects the pressor action of 10 μg. *nor*adrenaline (N).

Splenic sympathin

In seven experiments on single cats, stimulation of the splenic nerves for 45 sec. resulted in a small rise of blood pressure, a small contraction of the nictitating

membrane, but no action on the non-pregnant uterus. This result was equated with that produced by intraportal infusions of about 0.1 μg. *nor*adrenaline.

In six out of nine experiments, the result of injecting the blood taken from the splenic vein of one cat after stimulation into exposed arteries of another cat indicated that mainly *nor*adrenaline was liberated in a mean concentration of 0.25 μg./ml. blood (range 0.2–0.66 μg./ml.). Control blood samples showed no action on the blood pressure and uterus and only a trace on the nictitating membrane. As Peart (1949) reported, the stimulation caused an initial rapid increase of flow followed by a slowing.

Uterine sympathin

In a series of 16 cats under chloralose anaesthesia, it was first confirmed that adrenaline and *nor*adrenaline injected into the femoral vein produced relaxation of the non-pregnant uterus, and in certain cases (8 out of 14 observations) the *nor*-adrenaline response was facilitated by the previous injection of adrenaline (Fig. 3).

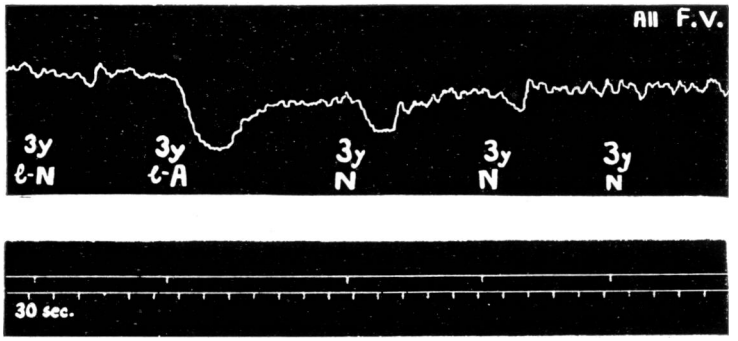

FIG. 3.—Cat. Chloralose. 1.4 kg. Non-pregnant uterus. The effect of intravenous adrenaline (3 μg. A) on the intravenous injection of *nor*adrenaline (3 μg. N).

When these amines were injected into the external iliac artery (so that the drug passed directly to the uterine tissue), both were inhibitory, their effects corresponding to the result of hypogastric nerve stimulation.

When pregnant cats were used, all three actions were excitatory, and ratio values for equiactive doses of the two amines were decreased, when compared with those

TABLE II

THE INFLUENCE OF THE SEXUAL STATE ON THE RESPONSE OF THE UTERUS TO EQUIACTIVE DOSES OF *nor*ADRENALINE (N) AND ADRENALINE (A), AND TO NERVE STIMULATION

Number of cats	Sexual state	Ratio N/A		Excitor (E) or Inhibitor (I) action	Hypogastric nerve stimulation E or I
		In vitro	*In vivo* Ex. I.A.		
1	Immature	25	—	I	I
14	Non-pregnant	8–10	15–20	I	I
2	Oestrus	5	2–10	I	I
6	Early pregnancy	2–5	1–3	E	E
3	Pregnancy	1–2	1–3	E	E
2	Late pregnancy	0.8–1	1–2	E	E

obtained with non-pregnant cats (Table II). In fact, towards late pregnancy *nor*-adrenaline was as active as adrenaline both *in vivo* and *in vitro* (West, 1947). So far it has not been possible to identify the sympathin in the uterine or ovarian veins after stimulation.

Discussion

During the last few years evidence has been accumulating to indicate that *nor*-adrenaline is liberated as a sympathetic transmitter. The work reported here has added further support to this conclusion. Blaschko (1942) suggested that the immediate precursor of adrenaline in the body may be the primary amine which would be converted to adrenaline by N-methylation, and hence it may be expected that a mixture of the amines might be liberated when the sympathetic nerves are stimulated. The exact proportion of each may be affected by the length of time the nerve has been sectioned before being stimulated (West, 1949).

After the first few experiments with single cats, we tried a cross-circulation technique. Cross-circulation was established in pairs of cats through glass cannulae which connected the cardiac end of the femoral artery of each animal with the cardiac end of the femoral vein of the other. The splanchnic, hepatic, or splenic nerves were stimulated in one cat and the effects of sympathin on the nictitating membrane and non-pregnant uterus of the recipient cat recorded. The results, however, were rather mixed, probably because of alterations in blood pressure, although the general indications were that mixtures of adrenaline and *nor*adrenaline were liberated.

The advantages of injecting the blood immediately after withdrawal from a stimulated cat into the arteries supplying the nictitating membrane and uterus of a second cat are: (1) the maximum concentration of sympathin in the blood reaches the recording tissues, loss on the way being eliminated ; (2) the actions recorded can be equated by intra-arterial injections of mixtures of the amines, given at the same speed in the same volume and washed in with the same volume of saline ; and (3) both animals are under chloralose anaesthesia. However, even then results may be misleading owing to localized fluctuations of blood pressure or of temperature. Some experiments were complicated by the release of interfering substances (Gaddum, Peart, and Vogt, 1949), and these may have been the cause of the inability to measure uterine sympathin by this technique. The results of splenic and hepatic sympathins have now been confirmed by direct measurement on the plasma and are reported in the next paper (Mann and West, 1950).

Summary

1. After stimulating certain sympathetic nerves in the cat, the blood has been withdrawn and injected into the arteries supplying the nictitating membrane and non-pregnant uterus of another cat in order to study the nature of four sources of sympathin.

2. When the splanchnic nerve is continuously stimulated, relatively more *nor*-adrenaline than adrenaline is liberated from the adrenal gland.

3. When the hepatic nerve is stimulated immediately after sectioning, the blood in the hepatic vein contains *nor*adrenaline and traces of adrenaline. Later stimulation appears to result in the liberation of *nor*adrenaline only.

4. When the splenic nerve is stimulated, the blood in the splenic vein contains *nor*adrenaline with traces of adrenaline.

5. It has not been possible to identify the sympathin in the blood of the ovarian vein when the hypogastric nerve is stimulated.

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