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THE ABSENCE OF AN EFFECT OF DIETARY PROTEIN ON THE LIBERATION OF LIVER SYMPATHIN AFTER STIMULATION OF THE HEPATIC NERVES

BY

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When Cannon and Uridil (1921) first showed that stimulation of the hepatic nerves liberated sympathin from the liver into the blood stream, they also noted that fasted cats appeared to set free less liver sympathin than cats which had recently been or were digesting meat. Cannon and Griffith (1922) found that, in general, stimulation of the hepatic nerves of cats fed on milk or meat led to a greater increase in the rate of the denervated heart than stimulation in cats fed mainly on fat or carbohydrate. There were, however, a number of cats which did not fit into this rule. When it became clear that the protein content of the diet had profound effects on the composition of the liver, viz., that the amount of liver cell cytoplasm varied directly with the quantity or quality of dietary protein (Addis, Lee, Lew, and Poo, 1940 ; Kosterlitz, 1947 ; Campbell and Kosterlitz, 1950, 1951), it was thought desirable to examine the possibility of a causal relationship between the results obtained by Cannon and his collaborators and the biochemical changes in the liver.

The evidence which has accumulated during the last few years has made it almost certain that the sympathin liberated after stimulation of the hepatic nerves is identical with *l*-noradrenaline (Gaddum and Goodwin, 1947 ; Mann and West, 1950 ; West, 1950). In their experiments, Cannon and Uridil (1921) and Cannon and Griffith (1922) were not in a position to match the increases in heart rate found after stimulation of the hepatic nerves with that obtained after known doses of *l*-noradrenaline. Therefore they were unable to exclude the possibility that their results were partly or wholly caused by a change in sensitivity of the denervated heart and not by a change in the quantity of sympathin or noradrenaline liberated. Evidence is presented in this paper that the variations in the increase in heart rate after stimulation of the hepatic nerves are due to such fortuitous variations in the sensitivity of the acutely denervated heart and not to effects of dietary protein.

METHODS

General design.—The amount of noradrenaline liberated after stimulation of the hepatic nerves is determined by its action on the chronically denervated nictitating membrane and on the rate of the acutely denervated heart, before and after cocaine.

It has been shown recently (Innes and Kosterlitz, 1950, 1951) that the effect of noradrenaline on the acutely denervated heart differs from that of adrenaline in that the chronotropic action of noradrenaline is usually, but not always, smaller than that of adrenaline. Furthermore, cocaine potentiates the chronotropic action of noradrenaline but not that of adrenaline. In the exceptional cases, when noradrenaline is more effective than adrenaline, the potentiating effect of cocaine is small or absent. In order to correlate the amount of noradrenaline liberated with the protein content of the diet, use is made of the fact that the protein content of a liver cell, expressed as the ratio liver protein N/deoxyribonucleic acid P (DNA-P) is a function of the quantity and quality of dietary protein (Kosterlitz, 1947; Campbell and Kosterlitz, 1950, 1951).

Operative.—In all cats in which the heart was to be denervated in the acute experiment (DH series) the right adrenal was removed and the left adrenal denervated in a preliminary operation performed under nembutal or nembutal-ether anaesthesia two to three weeks before the acute experiment. At the same time, the right nictitating membrane was postganglionically denervated by extirpation of the superior cervical ganglion. In cats in which the chronically denervated nictitating membrane was the sole indicator for liver sympathin (N series) the adrenals were removed during the acute experiment.

Experimental.—For one week before the acute experiments the cats were placed on either a low-protein diet (potatoes), the stock diet (meat, potatoes, and milk), or a high-protein diet (meat, fish, and milk).

Most of the acute experiments were performed under nembutal anaesthesia, but ether was used for a small number in order to imitate the conditions of Cannon and his collaborators as closely as possible. Both vagi were divided and the middle cervical ganglia removed. Then both stellate ganglia were excised by the antero-lateral approach through the first intercostal spaces. For the isolation of the hepatic nerves, the first step was the dissection and cannulation of the common bile duct. Then the portal vein was freed of surrounding tissue, the pancreatico-duodenal artery identified and divided together with any remaining tissue between the liver and the duodenum, and finally the hepatic nerves separated from the hepatic artery and cut as far centrally as possible.

The hepatic nerves were stimulated with shielded silver electrodes. The Ritchie-B.N.I. square wave stimulator (Walter and Ritchie, 1945) was used, and the stimulus, lasting 15 seconds, was almost always 20 V., 1 msec., 40 cyc./sec. The contractions of the nictitating membrane were recorded with an isotonic lever.

The arterial B.P. was measured in the femoral artery with a mercury manometer, heparin being used as anticoagulant. The heart rate was determined from the B.P. tracing for 30 seconds preceding the stimulation of the hepatic nerves (basal heart rate) and for 90 or 120 seconds after stimulation. The values are given in beats/min.

Stock solutions of the sympathomimetic drugs were prepared by dissolving *l*-adrenaline in 0.01 N-HCl, *dl*-noradrenaline hydrochloride and *l*-noradrenaline D-bitartrate monohydrate in water. All solutions contained 1 mg. base/ml. The final dilutions were made up with Ringer-Locke solution containing 1 mg. ascorbic acid/1 ml. The solutions containing the drugs were injected in quantities of 0.1–0.4 ml. into the femoral vein, the injection always taking 15 seconds. All values are given as μg. base.

It was usually not difficult to match the contractions of the nictitating membrane after stimulation of the hepatic nerves with those after injection of noradrenaline. This was, however, almost impossible for the chronotropic action, since the increases in heart rate could only be counted after the end of the experiment. For this reason the increase in heart rate after stimulation of the nerves was very often smaller than that after the smallest dose of noradrenaline. To give some idea of the quantity which would have matched in these instances, the ratio, $100\times$ increase in heart rate after stimulation of hepatic nerves to increase in heart rate after smallest dose of noradrenaline, is also

given in Table I. A similar procedure was adopted for the nictitating membrane, when necessary.

In all experiments in which the acutely denervated heart was used and in a few experiments with the chronically denervated nictitating membrane alone, the stimulations and the injection of the matching doses were repeated after the intramuscular injection of cocaine hydrochloride (8 mg./kg.).

Chemical.—At the end of the experiment samples of all liver lobes were removed, pooled, and a sample of 2 g. weighed for the estimation of deoxyribonucleic acid P (DNA-P) and protein N as described previously (Campbell and Kosterlitz, 1949, 1951).

TABLE I

THE EFFECT OF DIETARY PROTEIN ON THE AMOUNT OF *l*-NORADRENALINE LIBERATED AFTER STIMULATION OF THE HEPATIC NERVES

Exp.	Protein content of diet	Protein N/DNA-P	Approximate quantity of *l*-noradrenaline (μg.) liberated as estimated from the response of	
			Nictitating membrane	Denervated heart
DH 5	Low	76	<0.5 (70)	<0.5 (50)
N 60	Low	86	0.75–1.0	
N 42	Medium†	87	0.25–0.5	
N 69	Low	90	0.5	
DH 23	Low	91	0.6–1.1	0.6
DH 19	Low	97	<0.6 (70)	<0.6 (70)
DH 28	Low	101	<0.4 (30)	0.4
N 48	Low	105	0.5	
DH 14	Medium	105	<0.6 (70)	<0.6 (40)
N 46	Medium	106	0.13–0.25	
N 43	Medium	107	0.25	
N 62	High†	110	0.25	
N 36	Medium	113	0.5	
N 39	Medium	115	0.5	
N 52	High†	116	0.5	
N 35	—	121	1.0	
DH 16	Medium	123	0.6	0.6
DH 20	Medium	125	<0.6 (30)	<0.6 (10)
N 68	High	127	1.0	
N 55	High	128	0.25–0.5	
N 65	High	128	0.25	
DH 74*	High	134		<0.4 (60)
N 51	High	139	0.25–0.5	
N 54	High	139	0.5	
DH 81*	High	140	0.4	<0.4 (70)
DH 26	High	143	<0.4 (40)	<0.4 (70)
N 59	High	144	0.25	
DH 78*	High	144	0.4 (60)	<0.4 (60)
DH 27	High	144	0.4–0.8	0.4–0.8
DH 21	Hign	145	<0.6 (70)	<0.6 (50)
DH 22	High	154	<0.6 (40)	<0.6 (30)
DH 80*	High	158	0.8	0.4–0.8
DH 77*	High	168	0.4	<0.4 (50)
DH 67*	High	177	0.8	0.4

* In these experiments the anaesthetic was ether; in all other experiments nembutal (45 mg./kg.) was used.

† These animals were poor eaters.

The figures in parentheses are the ratios 100 × increase in heart rate after stimulation of the hepatic nerves/increase in heart rate after the smallest dose of *l*-noradrenaline. All doses are given as *l*-noradrenaline. When *dl*-noradrenaline was used for matching (in experiments with the code letter "N"), the dose of *dl*-noradrenaline was twice that given in the Table.

RESULTS

The protein content of a liver cell or rather of a unit of liver cells of the cat, expressed as the ratio protein N/DNA-P, increased with rising dietary protein intake (Table I) in the same way as was shown in the rat (Campbell and Kosterlitz, 1950). This ratio may therefore be used to indicate the amount of protein eaten.

The increase in heart rate after stimulation of the hepatic nerves varied considerably from experiment to experiment. Usually the increase was about 5 beats/min., quite independently of the protein content of the liver cells (Fig. 1). Although

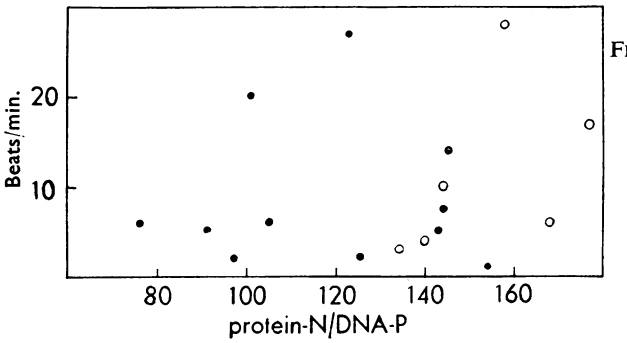

FIG. 1.—Increase in heart rate during 1½ min. after stimulation of hepatic nerves in cats with livers of varying protein content. The hepatic nerves were stimulated for 15 sec. with rectangular pulses, 20 V., 1 msec., 40 cyc./sec. The dots and circles indicate values obtained from cats under nembutal and under ether respectively.

the responses in 4 out of 16 cats (Exps. DH 16, 28, 67, 80) showed an increase of 15 or more beats/min., these were in no way correlated with high protein contents of the liver cells. Further, these responses were not due to the liberation of particularly large quantities of noradrenaline but to an increased sensitivity of the heart to amounts which usually caused increases in heart rate of rather less than 10 beats/min. The reason for the peculiar responses of these four cats is probably to be found in the fact that acute denervation alone fully sensitized the heart and thus led to a greater response than in the majority of cats. Cocaine had no potentiating effect in these cats (e.g., Fig. 2, Exp. DH 16).

There is a fair agreement between the results obtained with the nictitating membrane and those with the acutely denervated heart as indicator (Table I). It is obvious that there is no correlation between the amount of noradrenaline secreted after stimulation of the hepatic nerves and the protein content of the liver cells and therefore of the protein content of the diet. It should be noted that the cats with the highest protein N/DNA-P ratios were actively digesting fish or protein during the experiment. This is of importance, since Cannon and Griffith (1922) observed the greatest increases in heart rate in such cats.

A more detailed analysis is given of three experiments in each of which approximately 0.6–0.8 μg. of *l*-noradrenaline was secreted after stimulation of the hepatic nerves (Fig. 2). The protein content of the liver cells was relatively low in the first cat, approximately normal in the second, and high in the third. The increase in heart rate after stimulation of the hepatic nerves was small in the first and third cats but large in the second. Cocaine potentiated the small responses obtained after stimulation of the hepatic nerves and after injection of noradrenaline in cats 1 and 3, but not the large responses in cat 2. It was therefore impossible to correlate,

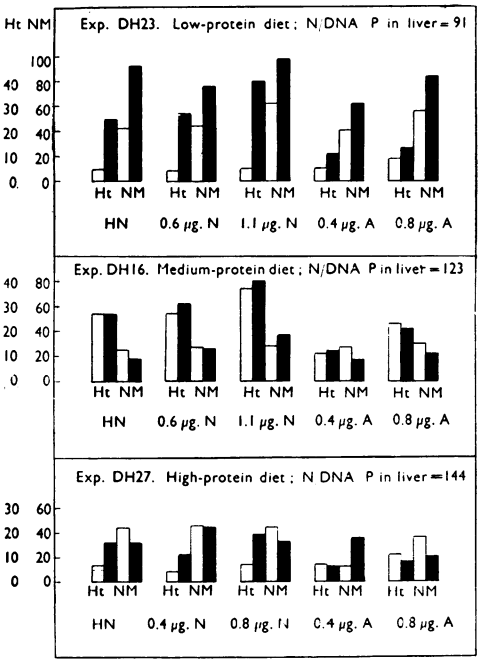

FIG. 2.—The effects of stimulation of the hepatic nerves and the injection of adrenaline and noradrenaline on the rate of the acutely denervated heart and on the chronically denervated nictitating membrane. The clear columns indicate effects before, and the shaded ones after, the intramuscular injection of cocaine hydrochloride (8 mg./kg.). Ht = increase in heart rate (beats/min.) during first 1½ min.; NM = maximal contraction of nictitating membrane in mm. of record. N = *l*-noradrenaline; A = *l*-adrenaline. HN = hepatic nerves stimulated.

first, the increase in heart rate with the amount of noradrenaline liberated, and, secondly, the quantity of noradrenaline secreted with the protein content of the liver cells, and thus with the amount of dietary protein ingested. As was to be expected, the responses of the denervated heart and the denervated nictitating membrane after stimulation of the hepatic nerves were matched more readily by injecting noradrenaline intravenously than by injecting adrenaline.

DISCUSSION

The experimental results reported in this paper make it impossible to uphold the views of Cannon and Uridil (1921) and Cannon and Griffith (1922) on the possible relationship between the protein content of the diet and the amount of sympathin or noradrenaline liberated after stimulation of the hepatic nerves. It would appear that Cannon and his collaborators were misled by their inability to match the chronotropic responses of the denervated heart against noradrenaline and thus exclude variations in sensitivity of the heart. Cannon and Griffith (1922) themselves stated that a number of cats did not fit into their hypothesis, cats which had been fed on a high-protein diet but did not show a marked increase in heart rate after stimulation of the hepatic nerves. These exceptions gain additional significance in the light of the results reported in this paper.

These conclusions have come as a disappointment to the present authors, who had hoped that the findings of Cannon and his collaborators might form the basis of an interpretation of the physiological significance of the changes occurring in the composition of the liver cell when the protein content of the diet is varied. Perhaps

Cannon himself doubted the validity of the earlier experiments when he and Lissák (1939) discussed the significance of the fact that the parenchymal cells of the liver did not receive sympathetic fibres.

Summary

1. The findings of Cannon and Uridil (1921) and Cannon and Griffith (1922), viz., that cats fed on a diet rich in protein liberate more sympathin on stimulation of the hepatic nerves than cats fed on diets poor in protein, have been re-examined.

2. The responses of the acutely denervated heart and of the chronically denervated nictitating membrane to stimulation of the hepatic nerves were matched with noradrenaline injected into the femoral vein. No correlation was found between the amounts of noradrenaline secreted and the amount of dietary protein eaten.

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