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EFFECT OF DENERVATION ON ENZYMES IN IRIS AND BLOOD VESSELS

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An investigation was described in 1952 by Burn and Robinson in which the effect of denervation on the amine oxidase present in the nictitating membrane, the iris and the blood vessels of the foreleg of the cat was studied. Denervation of the nictitating membrane and of the iris consisted in removal of the superior cervical ganglion and denervation of the foreleg arteries in removal of the stellate ganglion. The number of observations made on the nictitating membrane was large; these showed that in the period 9–12 days after removal of the ganglion the amine oxidase present diminished to about 60% of that on the normal side, but that during the next three weeks the amount increased once more until it was about the same as on the normal side.

The recovery of amine oxidase suggested that re-entry of sympathetic fibres into the nictitating membrane had taken place, most probably from fibres originating in the stellate ganglion and passing along the vertebral arteries. Burn and Robinson (1953) therefore carried out a second investigation in which both the superior cervical and the stellate ganglia were removed; they observed a fall in amine oxidase to a mean value of 50% of the normal, which persisted; the amount of amine oxidase did not recover as in the earlier experiments.

The denervated nictitating membrane has been shown to be hypersensitive in comparison with the innervated membrane to many agents other than adrenaline, and Burn and Trendelenburg (1954) re-investigated this question by perfusing the head of the cat. They found that the denervated membrane was regularly more sensitive than the normal membrane to adrenaline, noradrenaline and acetylcholine, but that both membranes were insensitive to other agents. They confirmed the evidence of Bacq and Fredericq (1935) that there are some cholinergic fibres in the sympathetic supply of the nictitating membrane. Thus the question arose whether the increased sensitivity of the denervated nictitating membrane to acetylcholine was due to a fall in cholinesterase. Burn and Philpot (1953) investigated this and found that both true and pseudocholinesterase were present in the nictitating membrane; they also found that in the denervated membrane there was a significant fall in true cholinesterase, but no similar fall in pseudocholinesterase. The observations thus combined to support the view that the hypersensitivity of the denervated nictitating membrane could be explained by a fall in the amount of the enzymes.

In the course of their work Burn and Philpot examined the cholinesterase in the iris after removal of the superior cervical ganglion. They expected that no change would be observed, but found that there was a fall in pseudocholinesterase. They pointed out the possibility that the hypersensitivity of the denervated iris to adrenaline might be related to this change rather than to the fall in amine oxidase described by Burn and Robinson (1952). A paper then appeared by Armin, Grant, Thompson and Tickner (1953) concerning the effect of denervation of the vessels of the rabbit ear. These authors found that degeneration of sympathetic fibres did not diminish the amine oxidase present in the vessels, but that it led to a reduction of the pseudocholinesterase to half the normal amount. They also showed that acetylcholine was present in the normally innervated vessel walls, and that this also disappeared after denervation. They suggested that the hypersensitivity to adrenaline was due to this disappearance.

While Burn and Robinson (1952) had investigated the change in amine oxidase in the denervated nictitating membrane very thoroughly, their evidence that a fall occurred in the denervated iris was much less strong, depending on only 7 experiments, the mean result being that the denervated membrane contained 77% of the activity of the normal membrane. The fall was conspicuous in only one experiment, and when this was omitted from the calculation the mean figure rose to 86%. Similarly, their conclusion that there was a fall in the amine oxidase in the denervated arteries was based (as they pointed out) on only 3 experiments. It was therefore decided to re-investigate the effect

of denervation on the amine oxidase in the iris and the arteries.

METHODS

For observations on the iris the amine oxidase of the iris of the right eye was compared with that of the left eye in 8 normal cats, and also in 8 cats in which the superior cervical ganglion of one side had been removed in an aseptic operation 8 or 9 days previously. Both eyes were removed from the orbit and the cornea was cut off. The iris was then lifted out with a pair of fine forceps, and, after lightly draining on a piece of cork, was weighed on a watch glass. In earlier experiments 2 cats were used for each estimation. The two irides from the same side were chopped finely with scissors and suspended in phosphate buffer M/15 *p*H 7.4 to give a volume of 1.1 ml. 0.5 ml. was used in each manometer vessel (vol. 6 ml.); in addition each vessel contained KCN 0.1M, 0.07 ml., H_2O 0.06 ml., KOH/KCN, 0.1 ml. (centre compartment), and in the side arm either 0.07 ml. H_2O or tyramine 0.1M, 0.07 ml. Substrate was added after 10–15 min. temperature equilibration; the gas was O_2, temperature 37.5° C. Readings were taken over 30 min. In later experiments the right and left irides from a single cat were compared; the control without tyramine, which was found to be negligible, was omitted. In these experiments each iris was minced with a McIlwain chopper and transferred completely to the manometer vessel.

Cat arteries were treated similarly. The piece of artery from each side was chopped and the whole transferred to the manometer vessel, phosphate buffer M/15 *p*H 7.4, 0.45 ml., was added and the other reagents as described above. Readings were taken over 90 min. No control measurement of tissue in absence of substrate was found to be necessary.

For estimation of pseudocholinesterase in arteries the tissue was treated exactly as has been described for the amine oxidase estimations. The whole of the piece of artery of one forelimb was compared with the whole of the piece of artery from the opposite limb. The artery was chopped finely with a McIlwain chopper and then transferred to the manometer flask. The flask contained 0.7 ml. Krebs bicarbonate Ringer solution. The side arm contained 0.08 ml. 0.2M-butyrylcholine chloride solution. A control flask, containing the Ringer and substrate only, measured the spontaneous hydrolysis of butyrylcholine chloride. Gas mixture was nitrogen containing 5% CO_2, temperature 37.5° C. Readings were taken over 30 min.

Observations were made in which the volume changes in the two forelegs of a cat were compared after intravenous injections of adrenaline and of noradrenaline. The cats were those in which the stellate ganglion of one side had been removed 8 or 9 days previously in a sterile operation, using Anderson's method (1904). The cats were first anaesthetized with ether and then a spinal preparation made. The volume changes were measured by putting the two forelegs in plethysmographs of the pattern described by Dale and Richards (1918), and the volume changes were recorded by piston recorders. These were sufficiently sensitive to show the pulse of the limb with each heart beat.

RESULTS

Amine Oxidase in Iris of Cat.—Results for the amount of amine oxidase in the normal and denervated iris are given in Table I. The figures for the right irides were expressed as a percentage of the corresponding figures for the left irides, and the mean percentage for normal right in terms of normal left was 98.4. When the figures for denervated right irides were expressed as a percentage of the corresponding figures for normal left irides, the mean percentage was 103.5. Thus the results indicated that removal of the superior cervical ganglion did not diminish the amount of amine oxidase in the iris.

TABLE I

AMINE OXIDASE IN IRIS EXPRESSED AS $\mu l.O_2/g./hr.$

Expt.	Normal Left	Normal Right	Denervated Right	Days Denervated	Right as % of Left
1	195	228	—	—	117
2	376	384	—	—	102
3	266	286	—	—	107
4	367	375	—	—	102
5	275	205	—	—	75
6	263	269	—	—	102
7	192	211	—	—	110
8	298	216	—	—	72
					Mean 98·4
9	224	—	270	8	120
10	331	—	427	8	129
11	410	—	375	9	91
12	325	—	246	9	76
13	164	—	252	9	154
14	294	—	274	9	93
15	370	—	321	9	87
16	426	—	342	9	80
					Mean 103·5

Amine Oxidase in the Foreleg Arteries.—Results for the amount of amine oxidase in the normal and denervated foreleg arteries are given in Table II. In the experiments in which the normally innervated vessels of the two forelegs were compared with one another the mean percentage for the right foreleg in terms of those of the left was 99.3. Similarly, the mean percentage for denervated vessels in terms of those of the corresponding normal vessels was 101.4 Thus the results indicated that removal of the stellate ganglion did not diminish the amount of amine oxidase in the foreleg arteries.

Pseudocholinesterase in the Foreleg Arteries.—As already described, Burn and Philpot found that extirpation of the superior cervical ganglion caused a fall in pseudocholinesterase in the iris, and Armin, Grant, Thompson and Tickner (1953) found that after denervation of the rabbit ear the

TABLE II
AMINE OXIDASE IN FORELEG ARTERIES OF CAT
μl.O_2/g./hr.

Expt.	Normal Left	Normal Right	Denervated Leg	Days Denervated	Result as % of Left or of Normal
17	162	170	—	—	105
18	176	181	—	—	103
19	105	94	—	—	90
					Mean 99·3
20	82	—	114	9	139
21	151	—	151	9	100
22	183	—	152	9	83
23	—	} 207	204	{ 8	} 98
24				8	
25	—	145	126	8	87
26		166	189	9	114
					Mean 103·5

pseudocholinesterase present in the vessel walls declined in amount and often disappeared. We therefore made estimations of pseudocholinesterase in the arteries of the cat's foreleg. A comparison was made of the amounts present in normal right and normal left legs, the results of which are shown in Table III, Expts. 27–32. The mean result in six experiments was that the amount in the right leg was the same as the amount in the left leg. A comparison was then made after denervating one leg by extirpation of the stellate ganglion. Eight experiments were carried out in which denervation

TABLE III
PSEUDOCHOLINESTERASE IN ARTERIES μl.CO_2/g./hr.

Expt.	Normal Left	Normal Right	Denervated Leg	Days Denervated	Result as % of Left or Normal
27	854	1,280	—	—	150
28	1,625	1,170	—	—	72
29	1,430	1,580	—	—	111
30	1,375	870	—	—	63
31	2,255	2,015	—	—	89
32	780	1,090	—	—	140
					Mean 104
33	—	1,975	2,420	8	123
34	1,110	—	1,215	8	107
35	—	1,445	1,395	8	96
36	—	1,770	1,067	11	60
37	577	—	1,670	11	290
38	1,040	—	1,370	14	132
39	—	1,750	1,940	29	110
40	1,000	—	1,295	33	130
					Mean 131

was performed 8–39 days previously. The results showed no loss of pseudocholinesterase on the denervated side, and the mean figure for the denervated legs was 131% of that for the normal legs. Since Armin, Grant, Thompson and Tickner found that the fall in pseudocholinesterase was greater when the time interval after denervation was longer we decided to carry out further experiments at a longer interval.

Denervation and Constrictor Effect of Adrenaline. —The denervated vessels of the rabbit ear were found by Armin, Grant and their colleagues to be much more sensitive to the constrictor action of adrenaline than the normal vessels. In 1932 Burn described the effect of removing the stellate ganglion on the constriction produced in the vessels of the cat's foreleg in response to injections of ephedrine and tyramine. He observed that the constrictor action of these substances was lost, but also that " the constrictor action of adrenaline was less in the denervated than in the normal limb." Figs. 5, 6 and 7 in that paper each show this difference, though it was not large.

We therefore compared the response to adrenaline of the normal and denervated forelegs by enclosing them in plethysmographs, the cat having been prepared as a spinal animal. An example is shown in Fig. 1. The upper record is of the volume of the normal leg, while the middle record is that of the denervated leg. When 10 μg. adrenaline was injected, the constriction in the normal leg seen as the downward movement of the record

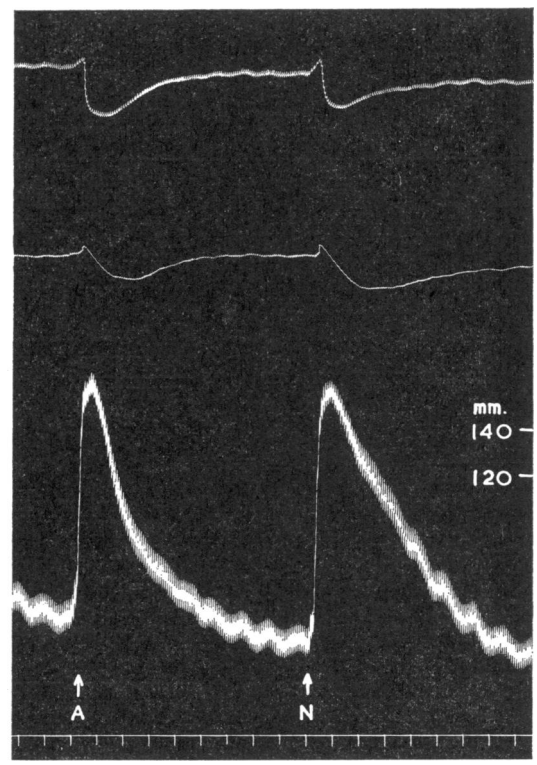

FIG. 1.—Upper record is the volume of the normal foreleg; middle record is the volume of the denervated foreleg; and lowest record is the blood pressure, in a spinal cat. The first injection is 10 μg. adrenaline, which caused more constriction in the normal foreleg than in the denervated. The second injection is 10 μg. noradrenaline, which caused about the same constriction in both forelegs.

was greater than that in the denervated leg. When 10 μg. noradrenaline was injected the constriction in the normal leg was about the same as that in the denervated leg.

In 10 cats in which the stellate ganglion of one side was removed from 35 to 55 days previously a series of injections of adrenaline and of noradrenaline was made, and the constrictions caused in the two legs were measured on the record afterwards. Mean values for these constrictions were then calculated, and the ratios of constriction in the normal leg to constriction in the denervated leg are shown in Table IV for both adrenaline and noradrenaline. At the end of each experiment the arteries were dissected and the pseudocholinesterase present was estimated. These results are also shown in Table IV together with the ratio of the amount present in the denervated leg to that in the normal leg. Thus the pseudocholinesterase ratios are expressed as $\frac{\text{denervated}}{\text{normal}}$, while the adrenaline constriction ratios are expressed as $\frac{\text{normal}}{\text{denervated}}$, for if the amount of pseudocholinesterase in some way restricts the adrenaline constriction then the ratios so expressed should show a correspondence.

TABLE IV

PSEUDOCHOLINESTERASE RATIOS AND CONSTRICTION RATIOS

Expt.	Pseudo ChE		ChE Ratio D/N	Constriction Ratio N/D	
	Normal	Denervated		Adrenaline	Noradrenaline
41	660	685	1·04	0·87	0·42
42	941	745	0·79	1·32	0·4
43	462	1,170	2·53	3·5	1·03
44	1,042	1,090	1·05	1·3	0·94
45	830	795	0·96	0·81	1·0
46	908	1,020	1·12	1·86	1·29
47	1,240	881	0·71	1·1	0·15
48	521	623	1·2	3·75	1·15
49	1,400	1,310	0·94	0·59	0·53
50	761	781	1·03	1·65	0·53
		Mean	1·14	1·67	0·74

Table IV shows figures for pseudocholinesterase similar to those in Table III and also that adrenaline caused more constriction in the normal leg than in the denervated in 7 of 10 experiments. The reverse was true of noradrenaline, which usually caused less constriction of the normal leg than of the denervated.

There appeared to be some degree of correlation between the amounts of pseudocholinesterase in the two legs and the responses to adrenaline. That is to say, the legs which constricted less in response to an injection of adrenaline were those in which the arteries were found to have more pseudocholinesterase.

When the figures in the fourth and fifth columns of Table IV were examined by the formula given by Fisher (1946), it was found that $r=0.6989$, $t=2.75$ and P was 0.025. Thus there was a correlation between the fourth and fifth columns of Table IV which was significant at the 2.5% level.

Observations with Enzyme Inhibitors.—Some observations have been made on the action of substances known to inhibit amine oxidase and cholinesterase. Marsilid (isopropylisoniazide) has been shown by Zeller and Barsky (1952) to inhibit amine oxidase and not to inhibit cholinesterase. Its effect was tested on the blood pressure and nictitating membrane of the spinal cat, and also in the perfused head of the cat (Burn and Trendelenburg, 1954). Injections of adrenaline and noradrenaline were made before and after injecting marsilid in amounts varying from 5 to 500 μg. The action of adrenaline on the blood pressure and on the nictitating membrane was not modified by these injections either in the spinal cat (4 expts.) or in the perfused head (5 expts.). In 2 of 5 experiments on the blood pressure the action of noradrenaline was increased after injecting marsilid, but these increases were much less than those regularly produced by the injection of cocaine.

The action of anticholinesterases was different. They were observed to diminish the pressor action of adrenaline and to increase its action on the nictitating membrane. An example is shown in Fig. 2 in which the injection of 0.8 mg. eserine sulphate had these effects. After the injection of

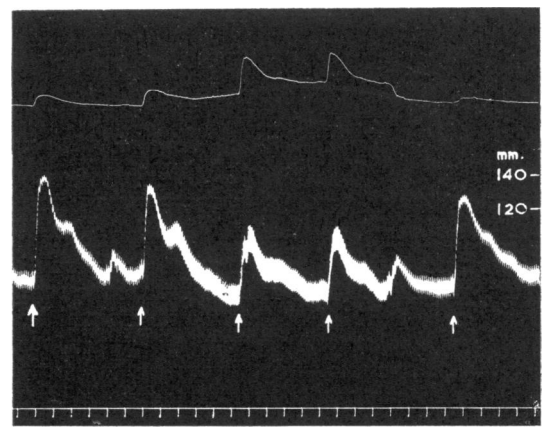

FIG. 2.—Upper record is the nictitating membrane, and the lower record is the blood pressure in a spinal cat. At each arrow 8 μg. adrenaline was injected. Between the first and second arrows 0.8 mg. eserine sulphate was injected, and between the fourth and fifth arrows 1.4 mg. atropine sulphate was injected.

1.4 mg. atropine sulphate the action of adrenaline on the nictitating membrane was almost abolished, while the pressor action was restored and sometimes increased. Neostigmine and Nu 683 (the dimethyl carbamate of (2 - hydroxy - 5 - phenyl - benzyl) - trimethylammonium bromide) were found to have a similar action to eserine. The amounts of these anticholinesterases which augmented the effect of adrenaline on the nictitating membrane were enough to cause fasciculation in skeletal muscle, but the augmentation was still evident after the injection of decamethonium up to 0.5 mg. to stop the fasciculation. When atropine was given first, in a dose of 2 mg., the augmentation was not seen.

Discussion

The observations have failed to confirm the previous work of Burn and Robinson (1952) that degeneration of the sympathetic supply leads to a fall in the amount of amine oxidase present in the iris and in the foreleg arteries of the cat. They came to the conclusion that such a fall took place on the basis of too few experiments because they had observed a fall in many experiments on the nictitating membrane.

The fact that amine oxidase in the iris is not affected by degeneration of the sympathetic fibres underlines the possibility mentioned by Burn and Philpot (1953) that the increased sensitivity to adrenaline caused by this degeneration depends on the fall in pseudocholinesterase which they recorded. This point needs further investigation in the isolated iris, in which the effect of anticholinesterases on the response to adrenaline could be studied.

In the foreleg arteries degeneration of the sympathetic fibres has not been found to cause a diminution either in the amount of amine oxidase or of pseudocholinesterase. A measurement has been made of volume changes in the normal and denervated forelegs both when adrenaline and noradrenaline were injected. Only in one of ten cats was the denervated foreleg more sensitive to the action of adrenaline than the normal foreleg, and in four it was much less sensitive. When the relative constriction in the two forelegs of each cat was compared with the relative amounts of pseudocholinesterase, it was found that there was a significant correlation between them.

These results point in the direction of the conclusions of Grant and Thompson and their colleagues that the response of blood vessels to adrenaline may be determined by the activity of a system in their walls which produces acetylcholine. If the constriction produced by adrenaline is limited by the acetylcholine present, then when there is much acetylcholine the constriction will be small. If the assumption is made that the activity of this acetylcholine system is proportional to the amount of pseudocholinesterase, then we have a basis for the correlation found between the constriction ratio of normal and denervated limbs in response to adrenaline and the ratio of the amounts of pseudocholinesterase present.

The effects of cholinesterase inhibitors in diminishing the pressor action of adrenaline and that of atropine in restoring it are also consistent with this explanation. So also is the potentiation of the action of adrenaline on the nictitating membrane by anticholinesterases, for both adrenaline and acetylcholine cause contraction of the nictitating membrane ; on this structure they do not oppose one another as in the blood vessels.

Griffin, Green, Youmans and Johnson (1954) have studied the changes in resistance which occur in the vessels of the dog's leg when adrenaline and noradrenaline are injected, comparing the results in normally innervated and previously denervated legs. They made observations on the muscle vessels and skin vessels separately. In the muscle vessels the effect of adrenaline was practically the same in the normal and previously denervated legs. The effect of noradrenaline was greater in the denervated legs, but the range of variation in the denervated legs was such that the difference was not statistically significant. In the skin vessels the effect of adrenaline was greater in the denervated legs by a significant amount. The effect of noradrenaline was also greater there, but again not significantly greater because of the wide spread. These findings appear to agree with the results of Armin, Grant, Thompson and Tickner in the rabbit ear vessels since these vessels are skin vessels, and they also agree with the results in the present paper since the volume changes recorded by the plethysmograph probably represent the reaction of muscle vessels. If this suggestion is correct it follows that denervated muscle vessels may not become more sensitive to adrenaline as do denervated skin vessels.

Burn and Robinson (1952) considered that the effect of cocaine in increasing the tone of the normal nictitating membrane, though not affecting that of the denervated membrane, was due to inhibition of amine oxidase. Since we have found that marsilid has no similar effect, it is doubtful whether their conclusion was correct. In view of Schayer and Smiley's evidence (1953) that the labelled carbon of the $-N.CH_3$ group was quanti-

tatively excreted in the urine of rats to which marsilid had previously been administered, it seems clear that a considerable proportion of adrenaline is destroyed in the body of the rat by amine oxidase, and we were therefore surprised to observe that marsilid did not potentiate effects of adrenaline. Smith and Alperi (1954) have studied the effect of amine oxidase inhibitors on isolated carotid arteries from swine and from dogs. They came to the conclusion that, while amine oxidase appears to play an important part in the arteries of swine, it has no importance in the arteries of the dog.

The effect of degeneration of sympathetic fibres on the response of the foreleg arteries to noradrenaline differed from that to adrenaline, since the denervated leg was more sensitive to noradrenaline in five of ten cats, and only in one was it rather less sensitive. This difference in the response of the two legs to noradrenaline cannot be related to such enzyme changes as we have observed, and must have some other explanation. We have none to put forward, but something about the difference between noradrenaline and adrenaline may be said.

Bülbring and Burn (1938) found that the response of the normal and denervated nictitating membranes varied for different sympathomimetic amines. The proportion of cats in which the denervated membrane responded less than the normal membrane to higher doses of these amines increased the more the structure of the amine differed from that of the transmitter. One of us (J. H. B.) suggested at the pharmacological meeting after the International Congress in Copenhagen (1950) that, while we think of the relation of stimulant substance and receptor as that of key and lock, it may be that normally the lock is capable of adjusting itself to many keys, but that after degeneration of the nerve supply it tends to lose this power of adjustment, and continues to be fitted perfectly only by the transmitter itself. Thus might be explained the diminished sensitiveness of the denervated foreleg arteries of many cats to adrenaline, while the sensitivity to noradrenaline remains greater than normal. This would agree with the observation (Burn, 1932) that tyramine failed to cause any constriction in the denervated limb. The hypersensitivity to noradrenaline, however, is not explained in these terms.

Summary

1. Further observations have been made on the effect of degeneration of sympathetic fibres on the amount of amine oxidase in the iris of the cat's eye and in the foreleg arteries of the cat. Degeneration of the sympathetic fibres does not cause a change in the amount of amine oxidase in either organ.

2. Degeneration of the sympathetic fibres to the cat's foreleg arteries does not reduce the amount of pseudocholinesterase present, nor does it make the foreleg more sensitive to the constrictor action of adrenaline.

3. In most experiments the constrictor response of the denervated foreleg to adrenaline was less than that of the normal foreleg, and the ratio of the two constrictor responses was found to be inversely related to the ratio of the amounts of pseudocholinesterase present.

4. The amine oxidase inhibitor, marsilid, did not modify the response of the nictitating membrane to adrenaline, nor the pressor response to adrenaline in the spinal cat. Cholinesterase inhibitors, however, diminished the pressor response to adrenaline and augmented the contraction of the nictitating membrane caused by adrenaline.

5. Degeneration of the sympathetic fibres to the cat's foreleg arteries usually makes the foreleg more sensitive to the constrictor action of noradrenaline.

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