On the Activities of the Medullary Extract of the Bovine Adrenal Gland Estimated by Some Biologic Tests, Using the Adrenaline-Noradrenaline Mixture as the Standard

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Comparisons of adrenaline estimates of the adrenal extract obtainable by some biologic and chemical tests were performed extensively by Kojima, Nemoto, Saito, Sato and Suzuki.¹⁾ Generally speaking, the estimates obtainable by various methods were not identical with each other. This disharmony of adrenaline estimates would be, as was suggested by Kojima *et al.*, due to the presence of some substances allied to adrenaline in the adrenal medulla.

Since L-noradrenaline was recently isolated by Bergström, Euler and Hamberg²⁾ as the crystalline base from the adrenal gland of cattle, the presence of noradrenaline in the bovine adrenals together with adrenaline will be unquestionable.

However, it is still uncertain whether active principles other than adrenaline and noradrenaline are contained in the adrenal medulla in a significant amount or not.

Is the fact that the different adrenaline estimates are obtained from the same adrenal extract according to the method applied, as was observed by Kojima *et al.*, wholly due to the presence of noradrenaline along with adrenaline in the adrenal medulla?

To clarify this problem, we have undertaken in the present investigation to compare the activities of the medullary extract of the bovine adrenals in some biologic tests, such as the rabbit intestine segment method, the dog blood pressure and the cat paradoxical pupil reaction, with that of the adrenaline-noradrenaline mixture.

EXPERIMENTAL

Methods

The medullary tissue of the bovine adrenals was separated from the cortical tissue and was extracted with 10% trichloroacetic acid. In the first place the extract was assayed chemically in terms of adrenaline and noradrenaline contents by the permanganate method of Suzuki and Ozaki³⁾. Then the biologic activities of the extract on the rabbit intestine segment method, the dog blood pressure and the cat denervated iris were estimated, using the adrenaline-noradrenaline mixture as the standard. The adrenaline-noradrenaline mixture was prepared so as to contain just the same percentage of noradrenaline and adrenaline with the chemical estimates of the bovine adrenal extract. L-adrenaline (Sankyo) and L-noradrenaline-D-bitartrate-mono-hydrate (Sterling Winthrop) were used. The amount of L-noradrenaline in 10 mg. of the latter was taken as 5 mg. These were dissolved in 1/10 N hydrochloric acid solution and were stocked in a refrigerator. Just before the tests, these stock solutions were further diluted with physiological saline solution and the mixture was prepared.

It may be needless to say that, if no other active principles than adrenaline and noradrenaline are contained in the adrenal medulla in a significant amount, the activities of the extract estimated by different biologic methods using the adrenaline-noradrenaline mixture as the standard will be similar each other.

The procedure of the biologic tests was wholly the same as was used in our previous investigation.⁴⁾

In the rabbit intestine segment method, the rhythmic movements of the intestine strip in the warmed diluted blood solution were recorded. Then the diluted blood solution containing a known quantity of the extract or the adrenaline-noradrenaline mixture was poured into the intestine chamber to replace the blood solution. Thus the activity of the adrenal extract on the movement of the isolated rabbit intestine segment was compared with that of the adrenaline-noradrenaline mixture.

In the test of the dog blood pressure, the transection of the spinal cord was performed at the lowest level of the cervical segments under ether anesthesia and two or three hours after the section the experiment was started without anesthesia. The diluted solution of the adrenal extract was injected intravenously.

In the tests of the cat denervated iris, the superior cervical ganglion was excised unilaterally under ether one week or more prior to the estimation. At the performance of the test, the animal was anesthetized with urethane. The diluted solution of the adrenaline-noradrenaline mixture or the adrenal extract was injected into the femoral vein.

Results and Discussion

With six specimens of the adrenal extracts, the estimations were performed. The results are summarized in Table I.

TABLE I
Comparison of Activities of the Medullary Extract of the Bovine
Adrenals, Estimated by Some Biologic Methods Using the
Adrenaline-Noradrenaline Mixture as the Standard

No. of extract	Chemical estimates of adrenaline and noradrenaline content				Biologic activities expressed in mg. equivalents of (adrenaline+ noradrenaline) per g. of medullary tissue		
	mg. per g. of medullary tissue			per cent noradre- naline	rabbit intest- ine segment method	dog blood pressure	cat parado- xical pupil reaction
	adren- aline	noradre- naline	adrenaline + nor- adrenaline				
1	6.7	2.6	9.3	28	11.9	11.1	9.9
2	5.4	3.2	8.6	37	11.3	9.1	10.1
3	8.6	3.6	12.2	30	12.8	13.6	14.7
4	8.5	3.5	12.0	29	17.0	11.3	15.8
5	7.6	3.0	10.6	28	10.4	10.4	12.4
6	6.2	3.2	9.4	34	11.1	11.1	11.1
Mean	7.2	3.2	10.4	31	12.5	11.2	12.4

Using the permanganate method of Suzuki and Ozaki, the noradrenaline was found to be contained 28–37% of the total active principles. The sum of the adrenaline and noradrenaline content estimated with this chemical method was 8.6–12.2 mg. per g. of the medullary tissue and the average value was 10.4 mg.

Using the adrenaline-noradrenaline mixture as the standard, the sum of adrenaline and noradrenaline content was estimated by the rabbit intestine segment method, the dog blood pressure method and the cat paradoxical pupil reaction. The rabbit intestine segment method and the cat pupil method yielded almost similar value, i.e. 12.5 mg. and 12.4 mg., respectively. The dog blood pressure method gave somewhat smaller value, i.e. 11.2 mg. If we compare the estimates obtained by three methods case by case, it will be noticed that the differences of values are rather small in all cases and may be accounted for by errors inherent to the methods tested, except for the extract No. 4.

From the experimental results above presented, it is justifiable to assume that the difference of the adrenaline estimates observed by Kojima *et al.* may be in the most of the cases due to the presence of noradrenaline together with adrenaline in the adrenal medullary extract.

Of the extract No. 4, peculiar values were obtained. In this case the

estimates obtainable by the rabbit intestine segment method and by the cat pupil method were 17.0 mg. and 15.8 mg., respectively, and were definitely larger than that given by the dog blood pressure method. The latter was 11.3 mg.

As the activity ratio of noradrenaline to adrenaline is the highest in the rabbit intestine segment method, the smallest in the cat paradoxical pupil reaction and the intermediate in the dog blood pressure,⁴⁾ the result obtained with the extract No. 4 can not be explained by the presence of simply two hormones in the adrenal medulla, i.e. adrenaline and noradrenaline.

In examining the experimental results of Kojima *et al.* concerning the adrenaline estimates of the bovine adrenal medullary tissue case by case, we were surprised to find that in Exps. No. 3 and No. 9 the ratios of the estimates obtainable by the three biologic methods were much the same as that in the extract No. 4 of the present investigation. In Exp. No. 3 of Kojima *et al.*, the adrenaline estimates obtained by the intestine segment method and the pupil method were 11.3 mg. and 11.2 mg., respectively, and that yielded by the blood pressure method was 7.4 mg. In Exp. No. 9, the estimate of adrenaline given by the intestine as well as by the pupil was 14.8 mg. and that yielded by the blood pressure was 9.6 mg. However, the standard solutions used in the experiments of Kojima *et al.* and of the present investigation are not the same. Adrenaline solution was used in the former and the adrenaline-noradrenaline mixture in the latter.

SUMMARY

The present investigation was designed with the desire to know whether the active principles other than adrenaline and noradrenaline are presented in the adrenal medulla in a significant amount or not.

The medullary tissue of the bovine adrenal gland was extracted with trichloroacetic acid. At first, the noradrenaline and adrenaline contents were estimated chemically by means of the permanganate method reported by Suzuki and Ozaki. The adrenaline-noradrenaline mixture was then prepared so as to contain just the same percentage of noradrenaline and adrenaline as the chemical estimates of the extract. Using this mixture as the standard the activity of the extract in three biologic tests was estimated quantitatively.

In five out of six specimens, the estimates obtainable by the three different methods were in every case almost similar each other. In one specimen, however, the estimate given by the blood pressure method was definitely smaller than those given by the intestine segment method and the pupil method.

Thus it can not yet be concluded that the active principles of the adrenal medulla are always solely two hormones, i.e. adrenaline and noradrenaline.

References

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