Brit. J. Pharmacol. (1951), 6, 120.

BIOLOGICAL ESTIMATION OF ADRENALINE AND *NOR*ADRENALINE IN TISSUE EXTRACTS CONTAMINATED WITH HISTAMINE OR ACETYLCHOLINE

BY

CARL G. SCHMITERLÖW*

From the Department of Pharmacology, Oxford University

(Received December 14, 1950)

Various biological methods have been devised for the estimation of adrenaline and *nor*adrenaline in tissue extracts. As these two substances frequently occur together in different proportions it has become necessary to adopt methods which allow the estimation not only of the total amount of pressor substances but also the percentage of adrenaline and *nor*adrenaline in a mixture. Two different ways have been followed. In the first, both these estimations are performed simultaneously and in the same animal, the cat's blood pressure being used as a test of the amount and the nictitating membrane or non-pregnant uterus *in situ* as an indicator of the percentage. The second way involves the use of two (or more) separate preparations, viz., cat's blood pressure and different isolated organs, such as rat's uterus, hen's rectal caecum, cat's intestine, etc. From the results thus obtained the percentage of adrenaline and *nor*adrenaline can be calculated, the principle being that the activity ratio adrenaline/*nor*adrenaline on the cat's blood pressure is correlated with the same ratio obtained from some test object on which the two substances differ significantly from each other in effect (see, e.g., West, 1947; Euler, 1949; Gaddum, Peart, and Vogt, 1949; Burn, Hutcheon, and Parker, 1950).

Both these ways involve the use of the cat's blood pressure for the estimation of the total pressor activity in tissue extracts. Various extraction methods have been adopted, and it should be remembered that figures given for the content of adrenaline and *nor*adrenaline in *tissues* are in fact only figures for the amounts estimated in *extracts* of these tissues. Many of these extracts contain histamine in addition to the pressor substances and this contamination may seriously interfere with the correct determination of the nature of these substances, as was pointed out by Schmiterlöw (1948a, b). Even if the amount of histamine is too small to exert any blood pressure lowering effect it may still cause a liberation of adrenaline from the adrenals of the test animal, and the effect of this released adrenaline is mixed with the effect of the extract itself.

This error can be avoided by tying off the adrenals of the test animal. But the admixture of blood pressure lowering substances may still lead to an erroneous

* With a grant from the Swedish Medical Research Council.

impression as regards both total amount and percentage of adrenaline and *nor*adrenaline, as will be shown here.

EXPERIMENTAL

Chloralosed cats.—The blood pressure and the contractions of the normal nictitating membrane were recorded in cats under chloralose (0.07 g. per kg. body weight). The animals were given cocaine in a dose of 3 mg./kg. intramuscularly at the beginning of an experiment in order to render them more sensitive to adrenaline and *nor*adrenaline.

Different mixtures of *nor*adrenaline and histamine were given in order to find the proportion in which the depressor effect of the histamine was completely masked by the pressor effect of the *nor*adrenaline. This mixture, giving a pure rise in blood pressure, caused a fairly strong contraction of the nictitating membrane. When these effects were compared with the action of the same amount of *nor*adrenaline given alone, two facts were observed. Firstly, the rise of blood pressure caused by the mixture was less than that caused by the *nor*adrenaline alone, although the difference was fairly small. Secondly, the mixture caused a much stronger contraction of the nictitating membrane. This is illustrated in Fig. 1.

The same experiment was carried out with mixtures containing *nor*adrenaline and acetylcholine. Similar results were obtained, the difference in response on the nictitating membrane between the mixture and the pure *nor*adrenaline being, however, less pronounced.

These experiments show that, if histamine or acetylcholine is present together with *nor*adrenaline, the latter substance cannot be accurately estimated by this method.

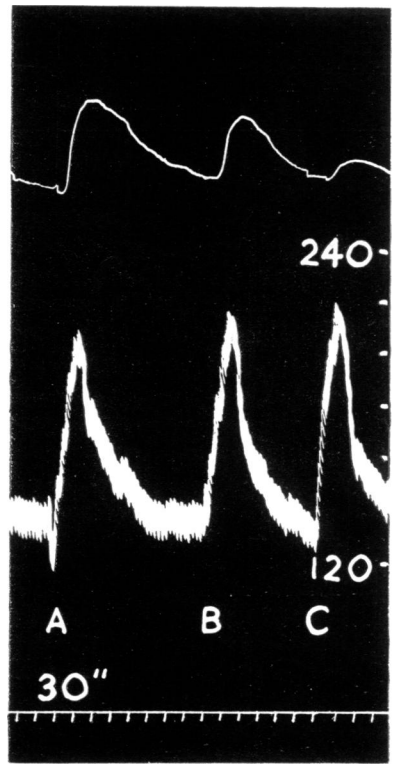

FIG. 1.—Chloralosed cat; cocaine. Normal nictitating membrane above, blood pressure below. A = 2 μg. *l-nor*adrenaline + 1 μg. histamine (base). B = 2 μg. *l-nor*adrenaline + 0.5 μg. histamine. C = 2 μg. *l-nor*adrenaline.

The following experiment was now performed:

1. Testing of the mixtures and pure *nor*adrenaline as described above.
2. Testing of the solutions after removal of the adrenals.
3. Testing after an antihistamine, and finally
4. Testing after atropine.

The results are illustrated in the figures. Fig. 2 shows the effects on a cat (under chloralose and after cocaine) before removal of the adrenals. The mixtures cause a less pronounced rise of blood pressure than *nor*adrenaline alone but a stronger contraction of the nictitating membrane than an equipressor dose of *nor*adrenaline (Fig. 2, D). Note that *nor*adrenaline plus histamine cause a slightly stronger contraction of the membrane than *nor*adrenaline plus acetylcholine. Histamine is obviously more active than acetylcholine in releasing adrenaline from the adrenals.

Fig. 3 (from the same cat as in Fig. 2) shows the effects after the adrenals had been removed. The mixtures now gave contractions of the nictitating membrane equal to that produced by the same amount of *nor*adrenaline alone, but they still gave a smaller rise in blood pressure.

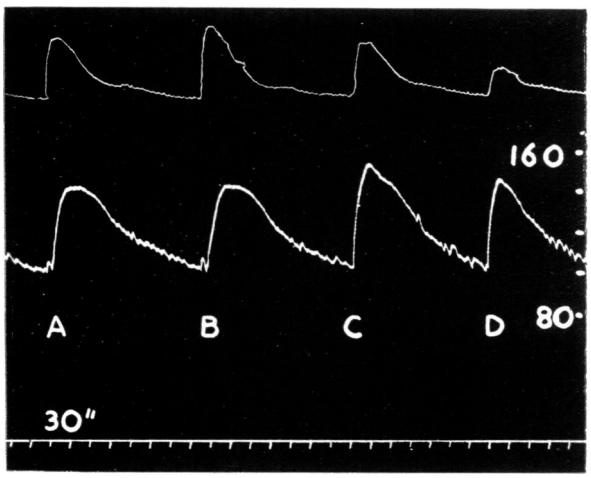

FIG. 2.—Chloralosed cat; cocaine. Normal nictitating membrane above, blood pressure below. A = 5.5 μg. *l-nor*adrenaline + 1 μg. acetylcholine. B = 5.5 μg. *l-nor*adrenaline + 1 μg. histamine. C = 5.5 μg. *l-nor*adrenaline. D = 3 μg. *l-nor*adrenaline.

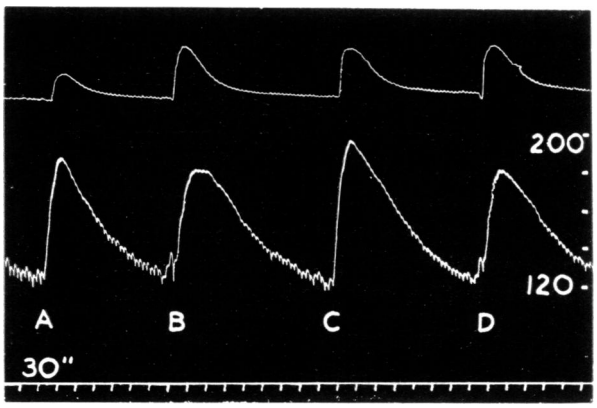

FIG. 3.—Same cat as in Fig. 2. Adrenals now removed. A = 3 μg. *l-nor*adrenaline. B = 5.5 μg. *l-nor*adrenaline + 1 μg. histamine. C = 5.5 μg. *l-nor*adrenaline. D = 5.5 μg. *l-nor*adrenaline + 1 μg. acetylcholine.

If an antihistamine (2 mg./kg. of Lergigan, described by Halpern and Schmiterlöw, 1951) was given the picture was altered. *Nor*adrenaline plus histamine now exerted the same action on both blood pressure and nictitating membrane as the same dose of

*nor*adrenaline alone, whereas *nor*adrenaline plus acetylcholine gave a smaller pressor effect. Finally atropine (0.5 mg. per kg.) was given, and Fig. 4 shows that the mixtures now behaved exactly as *nor*adrenaline alone, i.e., the *nor*adrenaline of the mixture could now be correctly estimated in spite of the admixture of histamine or acetylcholine.

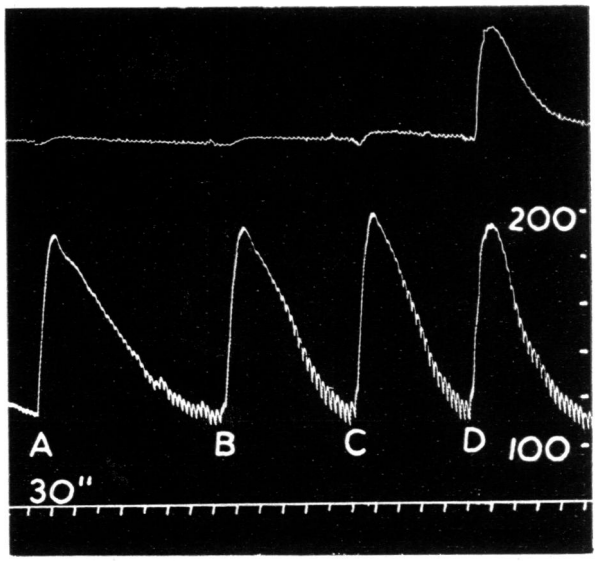

FIG. 4.—Same cat as in Figs. 2 and 3. Lergigan (2 mg./kg.) and atropine (0.5 mg./kg.) had now been given. A = 3 μg. *l-nor*adrenaline. B = 3 μg. *l-nor*adrenaline + 1 μg. histamine. C = 3 μg. *l-nor*adrenaline + 1 μg. acetylcholine. D = 7.5 μg. *l*-adrenaline.

Fig. 4 also reveals another interesting fact. Cocaine is well known to make the cat more sensitive to both adrenaline and *nor*adrenaline. The disadvantage of cocaine is that the normal nictitating membrane becomes sensitized to these substances in such a way that the difference between them becomes rather small, thereby reducing the possibility of using this organ for distinguishing between adrenaline and *nor*adrenaline. If, however, atropine is given the sensitivity of the membrane becomes altered, the action of adrenaline still being markedly potentiated whereas the sensitivity towards *nor*adrenaline is considerably decreased.

Spinal cats.—The effect of similar mixtures was tested on the blood pressure and the nictitating membrane of the spinal cat. In this preparation the admixture of small doses of histamine has a very marked effect on the pressor action of *nor*adrenaline. This is best illustrated in Fig. 5, in which it can be seen that 20 μg. *nor*adrenaline exerts a considerably stronger pressor effect than a mixture of 20 μg. *nor*adrenaline plus 1 μg. histamine, whereas the effect on the nictitating membrane is exactly the same (adrenals tied off). The same figure shows that if the histamine has been counteracted by giving an antihistamine the mixture becomes equiactive with pure *nor*adrenaline.

COMMENTS AND CONCLUSIONS

Adrenaline, *nor*adrenaline, histamine, and acetylcholine occur together in most tissues. When tissues are extracted it is natural that these substances may also occur in the extracts. When such extracts are tested biologically the effect of the pressor

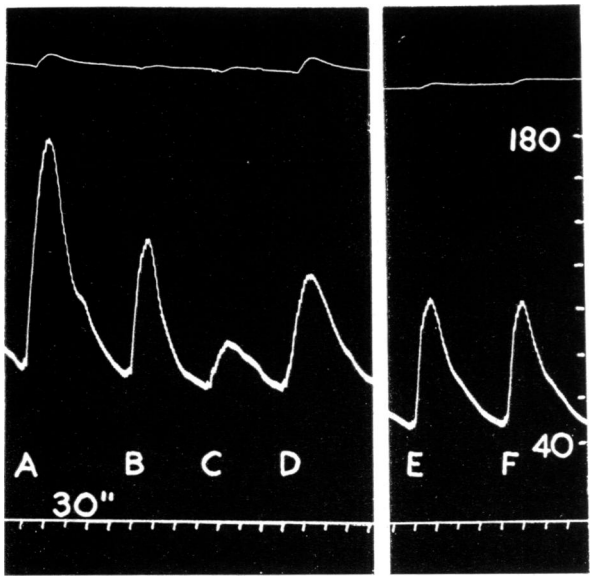

FIG. 5.—Spinal cat, adrenals removed. Normal nictitating membrane above, blood pressure below. A = 20 μg. *l-nor*-adrenaline. B = 10 μg. *l-nor*-adrenaline. C = 10 μg. *l-nor*-adrenaline + 1 μg. histamine. D = 20 μg. *l-nor*adrenaline + 1 μg. histamine. Between D and E Lergigan (2 mg./kg.) was given. E = 10 μg. *l-nor*-adrenaline + 1 μg. histamine. F = 10 μg. *l-nor*adrenaline.

substances may predominate to such an extent that the effect of the depressor substances is completely hidden.

When tissue extracts are tested on the cat's blood pressure and nictitating membrane with a view to estimating the amount of pressor substances, and simultaneously also the percentage of adrenaline and *nor*adrenaline, it must be remembered that contamination with even small amounts of histamine or acetylcholine may give rise to erroneous estimations. The reason for this is given in the present investigation.

1. The contaminating histamine and acetylcholine cause a release of adrenaline (or perhaps a mixture of adrenaline and *nor*adrenaline) from the suprarenals of the test animal itself.

2. These blood pressure lowering substances prevent the pressor substances from exerting their full action—they act as a braking mechanism.

The results of these two actions are the following. If adrenaline is released in the test animal the effects of this adrenaline will be added to the effects of the pressor substances in the extract, the effect of adrenaline being much stronger than *nor*adrenaline on the nictitating membrane (or on the non-pregnant uterus). The result of this adrenaline liberation will be interpreted in different ways, depending upon the nature of the pressor substance occurring in the extracts; three possibilities exist, viz., (*a*) if the extract contains *nor*adrenaline as the only pressor base the biological estimation may give the impression that there is in fact a mixture of adrenaline and *nor*adrenaline, (*b*) if the extract contains both adrenaline and *nor*adrenaline a false impression may be obtained about the percentage of each present, and (*c*) if the extract contains adrenaline as the only pressor base, estimation on the nictitating membrane may indicate a higher adrenaline content than that on the blood pressure, a result which might be interpreted as due to the presence of a pressor substance with a stronger effect on the nictitating membrane than adrenaline.

This release from the adrenals can, of course, be avoided if the adrenals are tied off. But even then contaminating histamine or acetylcholine may cause an error because of the second action mentioned above, viz., the braking mechanism. The pressor substances in the extracts cannot exert their full action on the blood pressure but will do so on the nictitating membrane or uterus, which are not affected by small doses of histamine or acetylcholine. The same arguments as those given above hold true even now. Let us assume that a tissue extract contains *nor*adrenaline and histamine and that 1 ml. of this extract, injected into the cat with adrenals tied off, is equipressor with 10 μg. *nor*adrenaline. On the nictitating membrane the extract exerts, however, a stronger contraction than the *nor*adrenaline. This finding suggests that the extract also contains adrenaline, whereas the real explanation is that 1 ml. of the extract actually contains 20 μg. *nor*adrenaline, the action of which was partly counteracted by the histamine on the blood pressure but not on the nictitating membrane. In other words, 1 ml. acted on the blood pressure like 10 μg. *nor*adrenaline, on the membrane it acted like 20 μg.

These errors exist when the biological estimation of amounts and percentages of adrenaline and *nor*adrenaline is performed on the same animal whether spinal or under chloralose. But even if separate preparations are used for these estimations, errors can still be made since the amount of pressor substances is still determined on the cat's blood pressure even if the percentage is estimated on isolated organs.

In order to avoid these erroneous estimations of pressor substances in extracts contaminated with histamine or acetylcholine the following precautions must be taken. The action of histamine and acetylcholine must be completely counteracted, which can be achieved by giving a suitable antihistamine and atropine. Furthermore, the adrenals should be tied off to exclude any possible liberation of pressor substances from them. The cat thus prepared is a very sensitive and reliable test object and can be used for the estimation of the total amount as well as percentage of adrenaline and *nor*adrenaline in a way analogous to the method described by Burn, Hutcheon, and Parker (1950).

A quite different way of avoiding the above-mentioned errors is to use extracts free from contaminating blood pressure lowering substances. Such an extraction method has recently been described by Euler (1948, 1949). In this method the pressor substances are adsorbed on to aluminium hydroxide, the blood pressure lowering substances remaining unadsorbed. This method of extraction has obviously a big advantage compared with other methods and should naturally be adopted when possible. Unfortunately it cannot always be used because the procedures involved take rather a long time.

Summary

1. Tissue extracts prepared for biological estimations of adrenaline and *nor*-adrenaline are often contaminated with histamine or acetylcholine. This admixture may cause a false impression of the amount and nature of the pressor substances, usually exaggerating the proportion of adrenaline.

2. Histamine and acetylcholine spoil the proper biological estimation of pressor substances in two ways:

(*a*) they release adrenaline from the adrenals of the test animal, and

(*b*) they prevent the pressor substances from exerting their full activity.

3. Special precautions must be taken when performing biological tests of such extracts.

4. A suitable preparation for this purpose is the cat under chloralose, treated with cocaine and given an antihistamine and atropine; the adrenals must also be tied off. If the blood pressure and the contractions of the normal nictitating membrane are recorded on this preparation adrenaline and *nor*adrenaline can be accurately estimated in spite of contaminating histamine or acetylcholine. The administration of atropine is essential for the discrimination between adrenaline and *nor*adrenaline.

5. If possible the extraction method described by Euler should be used. By this method the pressor substances are extracted but not the blood pressure lowering substances, the above-mentioned errors thus being avoided.

I am greatly indebted to Professor J. H. Burn for his great hospitality and for facilitating my work in this department.

References

Burn, J. H., Hutcheon, D. E., and Parker, R. H. O. (1950). *Brit. J. Pharmacol.*, **5**, 142.
Euler, U. S. von (1948). *Arch. int. Pharmacodyn.*, **77**, 477.
Euler, U. S. von (1949). *Acta physiol. scand.*, **19**, 207.
Gaddum, J. H., Peart, W. S., and Vogt, M. (1949). *J. Physiol.*, **108**, 467.
Halpern, B. N., and Schmiterlöw, C. G. (1951). To be published.
Schmiterlöw, C. G. (1948a). *Acta physiol. scand.*, **15**, 47.
Schmiterlöw, C. G. (1948b). *Acta physiol. scand.*, **16**, Suppl. 56.
West, G. B. (1947). *J. Physiol.*, **106**, 418.