The chromatography procedure was established after evaluating various sequences of different solvents and quantitatively determining the contents of 5-ml eluate fractions. Digoxigenin was recoverable in the first 25 ml of the 5% ethanol in chloroform eluate in six separate colorimetric determinations. Likewise, digoxin and its *mono-* and *bis*-digitoxosides were recovered in the first 10 ml of the 1:3 mixture (v/v) of 95% alcohol plus chloroform in six colorimetric determinations (Table 1).

In addition to the nonradioactive determinations, numerous extractions of tritiated digoxin from liver incubations have been performed in this laboratory.[5] Recoveries from 48 individual control experiments (both with and without tissue present) have yielded a mean of 95·62 ± 1·76 per cent of the original dose present in the flasks. The details of these metabolism studies will be made available in the near future.

Two precautions in regard to the chromatography procedure should be mentioned. We have restricted ourselves to the use of Alcoa F-20 alumina since certain other alumina preparations have been found to be unsuitable.* Likewise, care should be taken not to use chloroform that has been allowed to accumulate significant amounts of hydrochloric acid.

The method as outlined allows recovery of radiodigoxin and all metabolites except for volatile material and any metabolite not extractable from the original protein residue. The protein residue is readily available for radioactivity determinations by combustion techniques. The procedures as outlined are readily reproducible and relatively fast for the isolation of such chemically labile materials as the cardiac glycosides. It is stated that the further separation of digoxin from its *mono-* and *bis*-digitoxosides can be accomplished by paper chromatography using chloroform: isopropyl ether (9:1) on formamide-saturated paper.[6] As yet, we have not found this latter method completely satisfactory for quantitative separation when small amounts of drug are involved.

* Personal communication from G. T. Okita, Department of Pharmacology, University of Chicago, Ill., U.S.A.

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Effects of drugs on noradrenaline and 3-hydroxytyramine (dopamine) levels and on the noradrenaline to dopamine ratio in the rat brain

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The effects of centrally acting drugs on brain levels of 5-hydroxytryptamine,[1, 2] noradrenaline,[3, 4] dopamine[5] and γ-aminobutyric acid,[6] have recently been investigated in an attempt to explain their mode of action. Multiple injections of *dextro*-amphetamine lower brain noradrenaline levels in rats,[7] and rabbits,[8] while in cats *laevo*-ephedrine has no significant effect on the hypothalamic noradrenaline.[9] The experiments reported here were designed to determine whether the central

nervous system stimulant effects of amphetamine, and some related compounds, could be correlated with their effects on noradrenaline and dopamine levels in the rat brain.

The compounds investigated were *laevo*-ephedrine hydrochloride, *dextro*-amphetamine sulphate, *laevo*-amphetamine sulphate, *dextro*-methylamphetamine hydrochloride and optically inactive phenmetrazine hydrochloride (3-methyl-2-phenylmorpholine hydrochloride), and all doses reported refer to these salts. The drug solutions were prepared in 0·9% NaCl solution so that the required dose was contained in 0·2 ml/100 g body weight. Groups of two or three male rats (Wistar Strain) of approximately equal body weight (75 to 95 g), were used and the order in which the animals were injected intraperitoneally, killed by immersion in liquid nitrogen 3 hr after injection and the brains dissected out, extracted and eluted, was randomised using either a table of random numbers or a 3 × 3 Latin Square design.[10] 0·9% NaCl solution was used as the control.

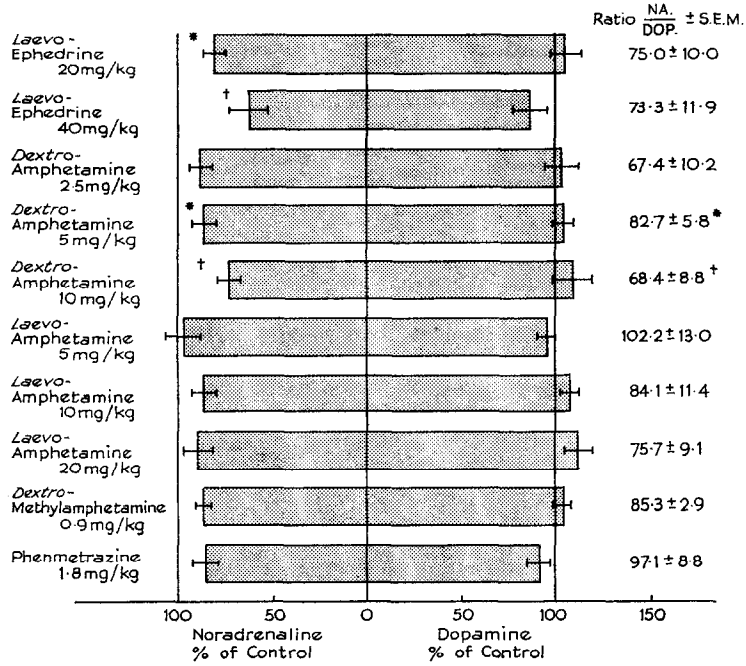

FIG. 1. The effects of drugs on the levels of noradrenaline and dopamine in the rat brain and on the ratio of noradrenaline to dopamine. All results are the means of 9 or 10 experiments and are expressed as percentages of control. The I-bar represents the Standard Error of the Mean (S.E.M.). The mean values for noradrenaline and dopamine, ±S.E.M., in the brains of 88 control animals were 0·65 ± 0·02 and 1·64 ± 0·06 μg/g respectively.

NA = noradrenaline, DOP = dopamine, * = $0{\cdot}05 > P > 0{\cdot}01$, † = $0{\cdot}01 > P > 0{\cdot}001$.

The brains were extracted with a total of 20 ml 0·4M perchloric acid as described by Lewis and Van Petten,[10] the noradrenaline and dopamine adsorbed on to acid-washed alumina at pH 8·5 and eluted with 2 vols. 5 ml 0·2N acetic acid.[11] Noradrenaline and dopamine were estimated spectrophotofluorimetrically by the methods of Bertler *et al.*,[12] and Carlsson and Waldeck[13] respectively.

Figure 1 shows the brain levels of noradrenaline and dopamine, expressed as percentages of control, in the drug-treated animals. Of the compounds investigated, only *dextro*-amphetamine (5 mg/kg and 10 mg/kg) and *laevo*-ephedrine (20 mg/kg and 40 mg/kg) produced falls in the

noradrenaline level which were statistically significant. In no case, however, was there any significant change in the dopamine level.

Since dopamine is the precursor of noradrenaline the effects of the drugs on the ratio, noradrenaline to dopamine, were studied, since a fall in this would perhaps indicate a decrease in noradrenaline synthesis. These ratios are shown in Fig. 1, expressed as percentages of control. Only *dextro*-amphetamine (5 mg/kg and 10 mg/kg), produced a significant change resulting in a fall in the ratio.

Both *laevo*-ephedrine (40 mg/kg), and optically inactive phenmetrazine (1·8 mg/kg) produced a significant fall in the total amount of noradrenaline and dopamine in the brain. None of the other drugs caused significant changes in the total amine content.

Goldstein and Contrera[14] have shown that optically inactive amphetamine and *p*-hydroxyamphetamine inhibit dopamine β-oxidase, which converts dopamine to noradrenaline. This might be expected to increase the level of dopamine and decrease that of noradrenaline, a suggestion which our results tend to confirm. They also support the proposal of Grana and Lilla[15] that there is no relationship between the ability of *dextro*-amphetamine to inhibit monoamine oxidase and its activity as a central nervous system stimulant.

Taking the effect on the noradrenaline level in the rat brain as an indication of potency, it would appear that *dextro*-amphetamine, since it is effective at a lower dose, is more potent than *laevo*-ephedrine, which is more potent than *laevo*-amphetamine. The comparative positions of *dextro*-methylamphetamine and optically inactive phenmetrazine are more difficult to assess, since they have been examined at only one dose level so far. Our findings agree with those of Schulte *et al.*,[16] who studied the central stimulant activity of a number of sympathomimetic amines by measuring the increase in spontaneous activity they produced in rats.

Thus for *dextro*- and *laevo*-amphetamine and *laevo*-ephedrine there seems to be some correlation between their central nervous system stimulant potency and their effects on the noradrenaline level in the rat brain. No such correlation exists when their effects on the dopamine level are considered.

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