This concept may help to relate the antisecretory (Anderson, Marcus & Watt, 1962; Eagleton, Watt & Marcus, 1968) and antipeptic actions of SGS in respect of their anti-ulcer action.

I thank Miss J. E. Harthill for technical assistance and Dr. H. G. Hind, Evans Biological Institute, for heparin and chondroitin sulphate.

*Department of Pharmaceutical Technology,*
*University of Strathclyde,* W. ANDERSON
*Glasgow, C.*1, *Scotland.*

January 31, 1969

REFERENCES

ANDERSON, W. & BAILLIE, A. J. (1967). *J. Pharm. Pharmac.*, **19**, 720–729.

ANDERSON, W., MARCUS, R. & WATT, J. (1962). *Ibid.*, **14**, *Suppl.*, 119*T*–121*T*.

DAVENPORT, H. W. (1965). *Gut*, **6**, 513.

EAGLETON, G. B., WATT, J. & MARCUS, R. (1968). *J. Pharm. Pharmac.*, **20**, 970–972.

GERALD, A., DE GRAEFF, J., LEV, R. & GLASS, G. B. J. (1967). *Proc. Soc. exp. Biol. Med.*, **124**, 1070–1073.

HAKKINEN, I., HARTIALA, K. & LANG, H. (1966). *Acta physiol. scand.*, **66**, 333–336.

HERRIOTT, R. M. (1938). *J. gen. Physiol.*, **21**, 501–540.

HERRIOTT, R. M. (1941). *Ibid.*, **24**, 325–338.

LONG, C. (1961). *Biochemists Handbook*. London: Spon.

NORTHROP, J. H., KUNITZ, M. & HERRIOTT, R. M. (1948). *Crystalline Enzymes*, 2nd edn. New York: Columbia University Press.

OVERHOLT, B. F. & POLLARD, H. M. (1968). *Gastroenterology*, **54**, 182–188.

SCHRAGER, J. (1964). *Nature, Lond.*, **201**, 1220–1222.

SPICER, S. S. (1965). *J. Histochem. Cytochem.*, **13**, 211–219.

SPICER, S. S. & SUN, D. C. H. (1967). *Ann. N. Y. Acad. Sci.*, 762–783.

## Amphetamine-induced release of [$^3$H]metaraminol from subcellular fractions of the mouse heart

The mode of action of amphetamine is complex and there are many studies reporting various sites of attack for this drug.

Amphetamine has a cell membrane pump blocking action—but it is not as potent as for example protriptyline (Carlsson & Waldeck, 1965; Malmfors, 1965; Carlsson, Lindqvist & others, 1965). It has also been suggested that amphetamine in large doses has a direct releasing effect on the amine storing granules. More recently Carlsson, Lindqvist, & others (1966a) suggested that amphetamine in low doses might act by displacement of amines from hypothetical extragranular binding sites or by an effect on the cell membrane leading to increased release (Carlsson, Fuxe & others, 1966b).

The aim with the present work was to see if studies on subcellular amine distribution could further elucidate the mode of action of amphetamine. This approach has proved useful for clarifying the mechanism of action of other drugs influencing adrenergic mechanisms (Lundborg, 1967). As in much of our previous work [$^3$H]-metaraminol, a noradrenaline analogue resistant to both monoamine oxidase and catechol-*O*-methyl transferase, was used.

Mice in groups of six, were given [$^3$H]metaraminol, 0·04 mg/kg, intravenously. Control groups received no further treatment and were killed by decapitation 30 min later. Other groups were injected with (+)-amphetamine bitartrate 5, 1 or 0·2 mg/kg (calculated as the salt) 15 min after the [$^3$H]metaraminol administration and were killed 15 min later. All animals were kept at an ambient temperature of 30°.

The hearts were removed and homogenized in the cold, using a plastic pestle, in 0·25 M sucrose containing 0·005 M phosphate buffer at pH 7·4 and 0·001 M $MgCl_2$. A coarse fraction was obtained by centrifugation at 4° at 2000 *g* for 10 min. The supernatant was then centrifuged at 100,000 *g* for 60 min in a Spinco model L Ultracentrifuge providing two more fractions, particulate (P) and high speed supernantant (S). After protein precipitation of the various fractions the samples were passed through an ion-exchange column (Dowex 50 W × 4). After elution the samples were analysed by means of liquid scintillation. Further details of the analytical procedure has been previously described (Stitzel & Lundborg, 1967).

When used in a dose of 5 or 1 mg/kg, (+)-amphetamine caused a pronounced decrease in the total content of [$^3$H]metaraminol in the mouse heart (Table 1). In a dose of 0·2 mg/kg the drug did not significantly change the [$^3$H]metaraminol content of the heart.

Table 1. *Effect of (+)-amphetamine bitartrate on the content and subcellular distribution of [$^3$H]metaraminol in the mouse heart.* The animals were killed 30 min after intravenous administration of 0·04 mg/kg of [$^3$H]metaraminol and (+)-amphetamine was given 15 min before death. The doses of (+)-amphetamine bitartrate are calculated as the salt. P/(P + S) × 100 means the amount of [$^3$H]metaraminol in the particulate fraction as a percentage of [$^3$H]metaraminol in the particulate + supernatant fractions.

| (+)-Amphetamine dose used mg/kg | No. of exp. | [$^3$H]Metaraminol ng/g | Significance test (Probability) | $\frac{P}{P+S} \times 100$ | Significance test (Probability) |
|---|---|---|---|---|---|
| 5 | 5 | 61·9 ± 4·8 | < 0·001 | 17·9 ± 1·0 | < 0·025 |
| — | 5 | 100·8 ± 12·5 | | 21·7 ± 1·1 | |
| 1 | 6 | 63·3 ± 1·7 | < 0·025 | 21·9 ± 1·0 | > 0·1 |
| — | 6 | 80·7 ± 5·2 | | 23·3 ± 1·9 | |
| 0·2 | 6 | 74·1 ± 1·8 | > 0·1 | 23·6 ± 1·6 | <0·01 |
| — | 6 | 78·7 ± 3·0 | | 17·1 ± 0·4 | |

At the 5 mg/kg dose, (+)-amphetamine caused a pronounced decrease in [$^3$H]-metaraminol levels in all three fractions of the heart (Fig. 1). The decrease was more prounced in the particulate (P) (50%) than in the supernatant (S) fraction (35%), resulting in a decrease in the P/(P + S) ratio (Table 1). Also, 1 mg/kg of (+)-amphetamine caused a decrease in the [$^3$H]metaraminol levels, though now of about the same magnitude in all three fractions. Thus the P/(P + S) ratio did not change significantly. After 0·2 mg/kg of (+)-amphetamine, the [$^3$H]metaraminol content was unchanged in the coarse fraction, increased in the particulate and decreased in the supernatant fraction. The P/(P + S) ratio was significantly increased.

These data imply that the dose used is of importance for the mode of action of (+)-amphetamine. In a dose of 5 mg/kg, (+)-amphetamine caused a release from the particulate (granular) fraction as well as from the supernatant fraction. The fact that the P/(P + S) ratio was decreased suggests that, under these conditions, the effect of (+)-amphetamine on the granular fraction is the dominating effect. Also in a dose of 1 mg/kg, (+)-amphetamine had a releasing effect on the granular fraction; however in these experiments this was of the same magnitude as the supernantant fraction. More interesting is the finding that in the low dose, 0·2 mg/kg, (+)-amphetamine bitartrate did not cause any release from the granules but induced a release of [$^3$H]metaraminol from the supernatant fraction only. This ability to release [$^3$H]metaraminol exclusively from the supernatant fraction has previously been observed for protriptyline, a potent membrane pump blocking agent (Lundborg & Stitzel, 1967). It is, however, doubtful if (+)-amphetamine in the low dose (0·2 mg/kg of the bitartrate) caused release of the [$^3$H]metaraminol by blockade of the cell membrane pump. In fact, the

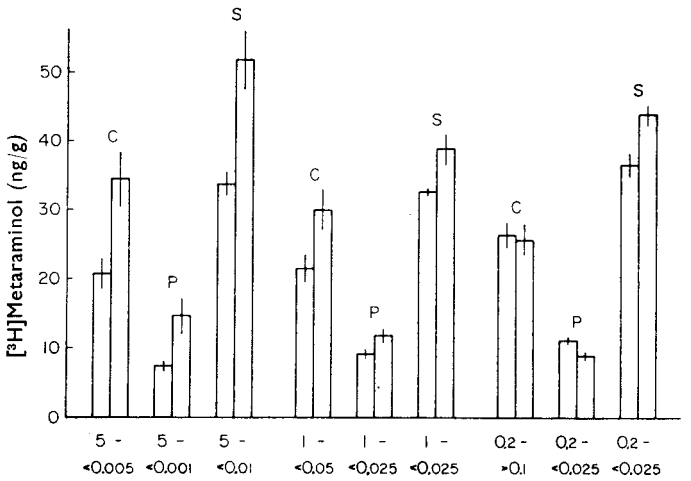


FIG. 1. Effect of (+)-amphetamine on the content of previously administered [³H]metaraminol in subcellular fractions (C = coarse fraction, P = particulate fraction, S = supernatant fraction) of the mouse heart. The dose of [³H]metaraminol was 40 μg/kg. The animals were injected with (+)-amphetamine 15 min after [³H]metaraminol and were killed after another 15 min. Vertical lines indicate standard error of the means. Abscissa: upper scale = amphetamine bitartrate (mg/kg); lower scale = probability.

membrane pump blocking effect of (+)-amphetamine can hardly be seen in this low dosage (Carlsson & others, 1966b and unpublished data). Furthermore, it is well-known that the catecholamine-releasing activity of (+)-amphetamine exceeds that of protriptyline although the latter agent is much more potent in blocking the membrane pump (Carlsson & Waldeck, 1965). This fact also indicates that the mode of action of amphetamine is different from that of protriptyline.

The present data confirm earlier suggestions by Carlsson & others (1966) and Fuxe & Hökfelt (1968) that (+)-amphetamine releases monoamines from extragranular binding sites. The present experimental procedure made possible the demonstration of this type of release more directly and without reserpine pretreatment.

The research reported in this manuscript has been supported by the Swedish State Medical Research Council (Grant Nos. B69-14X-2157-03A, B69-14X-2464-02). The highly competent technical assistance of Miss Lena Ramstedt is gratefully acknowledged.

*Department of Pharmacology*, PER LUNDBORG
*University of Göteborg*,
*Fack*, 400 33 *Göteborg* 33,
*Sweden*.

February 7, 1969

REFERENCES

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966b). *Acta physiol. scand.*, **67**, 481–497.

CARLSSON, A., LINDQVIST, M., DAHLSTRÖM, A., FUXE, K. & MASUOKA, D. (1965). *J. Pharm. Pharmac.*, **17**, 521–524.

CARLSSON, A., LINDQVIST, M., FUXE, K. & HAMBERGER, B. (1966a). *Ibid.*, **18**, 128–130.

CARLSSON, A. & WALDECK, B. (1965). *Ibid.*, **17**, 243–244.

FUXE, K. & HÖKFELT, T. (1968). *Europ. J. Pharmac.*, **4**, 135–144.

LUNDBORG, P. (1967). *Acta physiol. scand., Suppl.*, 302.

MALMFORS, T. (1965). *Ibid.*, **64**, *Suppl.* 248.

STITZEL, R. & LUNDBORG, P. (1967). *Br. J. Pharmac. Chemother.*, **29**, 342–349.