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Effect of reserpine on the subcellular distribution of ^{3}H-α-methylnoradrenaline in the mouse heart

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1. Mice were injected with reserpine (0·5 mg/kg or 2·5 mg/kg) at various intervals after an intravenous injection of ^{3}H-α-methylnoradrenaline (^{3}H-α-MeNa), 20 μg or 100 μg/kg. The effect of reserpine on the content of labelled amine in the subcellular fractions of the heart was studied.

2. In most experiments reserpine caused a pronounced decrease in the ^{3}H-α-MeNA content of the particulate fraction (P). The decrease was most pronounced when the higher dose of reserpine and lower dose of α-MeNA were used. In most experiments, the ^{3}H-α-MeNA content of the supernatant fractions was unchanged and, therefore, the P/(P + S) ratio was decreased.

3. It is concluded that the effect of reserpine is a releasing effect on the particulate (granular) fraction, probably by blocking the re-uptake of the amine leaked from the granules. No support was found for the hypothesis that reserpine acts by inhibition of release of amines from the nerve granules.

Several authors have shown that *in vitro* reserpine is a potent inhibitor of the specific ATP-Mg^{++}-sensitive uptake of catecholamines and 5-hydroxytryptamine by adrenal medullary granules (Carlsson, Hillarp & Waldeck, 1962; Kirshner, 1962a, b), and granules isolated from bovine splenic nerves (Euler & Lishajko, 1963a). It has also been shown that reserpine in low concentrations inhibits the spontaneous release of noradrenaline (NA) from isolated splenic nerve granules (Euler & Lishajko, 1961, 1963b), and it has been suggested that inhibition of release rather than block of uptake is the true effect of reserpine *in vivo* (Stjärne, 1966).

It has previously been shown that pretreatment with reserpine considerably inhibits the *in vivo* uptake of ^{3}H-noradrenaline (Stitzel & Lundborg, 1967) and ^{3}H-α-methyl-noradrenaline (Carlsson, Lundborg, Stitzel & Waldeck, 1967) by the amine-containing particles of the mouse heart. It has also been shown that reserpine causes release of previously administered ^{3}H-metaraminol from the particulate fraction of the mouse heart (Lundborg & Stitzel, 1967a). In contrast, Johnson (1964), administering reserpine to rats, was unable to find any change in the subcellular distribution of endogenous noradrenaline in the heart.

The affinity of metaraminol for granular binding sites is not the same as that of noradrenaline or α-methyl-noradrenaline. It was, therefore, desirable to study the releasing effect of reserpine on one of the latter amines. It is difficult to perform experiments of this kind with noradrenaline because this amine is a substrate for

monoamine oxidase and cannot accumulate in the cytoplasm outside the granules. For this reason α-methyl-noradrenaline, which is resistant to monoamine oxidase, was chosen for the present experiments.

Methods

All experiments were performed on female mice. The experiments were carried out at an ambient temperature of 30° C to avoid a fall in body temperature in the reserpine-treated mice.

The mice, divided into groups of six, were given racemic ^{3}H-α-methyl-noradrenaline (^{3}H-α-MeNA), 20 μg/kg or 100 μg/kg (100 mc/m-mole), intravenously. Control groups received no further treatment and were killed 30 min, 1 hr or 24 hr later. The experimental groups were injected with reserpine (0·5 mg/kg or 2·5 mg/kg) intravenously 15 min or 23 hr 15 min after ^{3}H-α-MeNA administration and killed 15 min or 45 min later.

The animals were killed by decapitation. The hearts were removed and homogenized at 0° C in a glass homogenizer by means of a plastic pestle. The medium was 0·25 M sucrose solution containing 0·001 M $MgCl_2$ and 0·005 M phosphate buffer, pH 7·4. A coarse fraction was obtained by centrifugation of the homogenates at 0° C at 2,000 *g* for 10 min. The supernatant obtained was then centrifuged in a Spinco Model L ultracentrifuge; this provided two more fractions, particulate (sediment) and high speed supernatant. After protein precipitation, the various fractions were placed on an ion exchange column (Dowex 50 W X4) and analysed as described previously (Carlsson *et al.*, 1967).

Tritium-labelled (±)-α-MeNA was prepared in cooperation with Hässle Ltd., Göteborg (Hallhagen & Waldeck, 1968). By the method described in this paper the tritium label should be in the positions 1′ and 2′, but it cannot be excluded that tritium will be introduced into other sites in the molecule by exchange. Reserpine was generously supplied by Swedish Ciba Ltd., Stockholm, Sweden. Statistical analysis was performed according to Winer (1962).

The significance levels of *P* adopted in this paper are: more than 0·1, not significant; 0·1–0·05, possibly significant; 0·05–0.01, significant; <0·01, highly significant.

Results

Release by reserpine of ^{3}H-α-MeNA from subcellular fractions of the mouse heart

Reserpine administration (0·5 mg/kg) 15 min before death caused a decrease in the ^{3}H-α-MeNA content of the coarse ($P<0·1$) and particulate ($P<0·05$) fractions from animals examined 30 min after the injection of ^{3}H-α-MeNA (Fig. 1). The supernatant fraction was unchanged.

Sixty minutes after the injection of ^{3}H-α-MeNA and 45 min after administration of reserpine the amount of the labelled amine in the particulate fractions was significantly decreased when the larger dose of reserpine (2·5 mg/kg) was used. There was a slight but significant ($P<0·01$) decrease in the supernatant fraction when the smaller dose (20 μg/kg) of ^{3}H-α-MeNA and larger dose (2·5 mg/kg) of reserpine were used. In all other cases the supernatant fraction was unchanged. The coarse fraction was decreased in all cases except when the smaller doses of reserpine and the labelled amine were given.

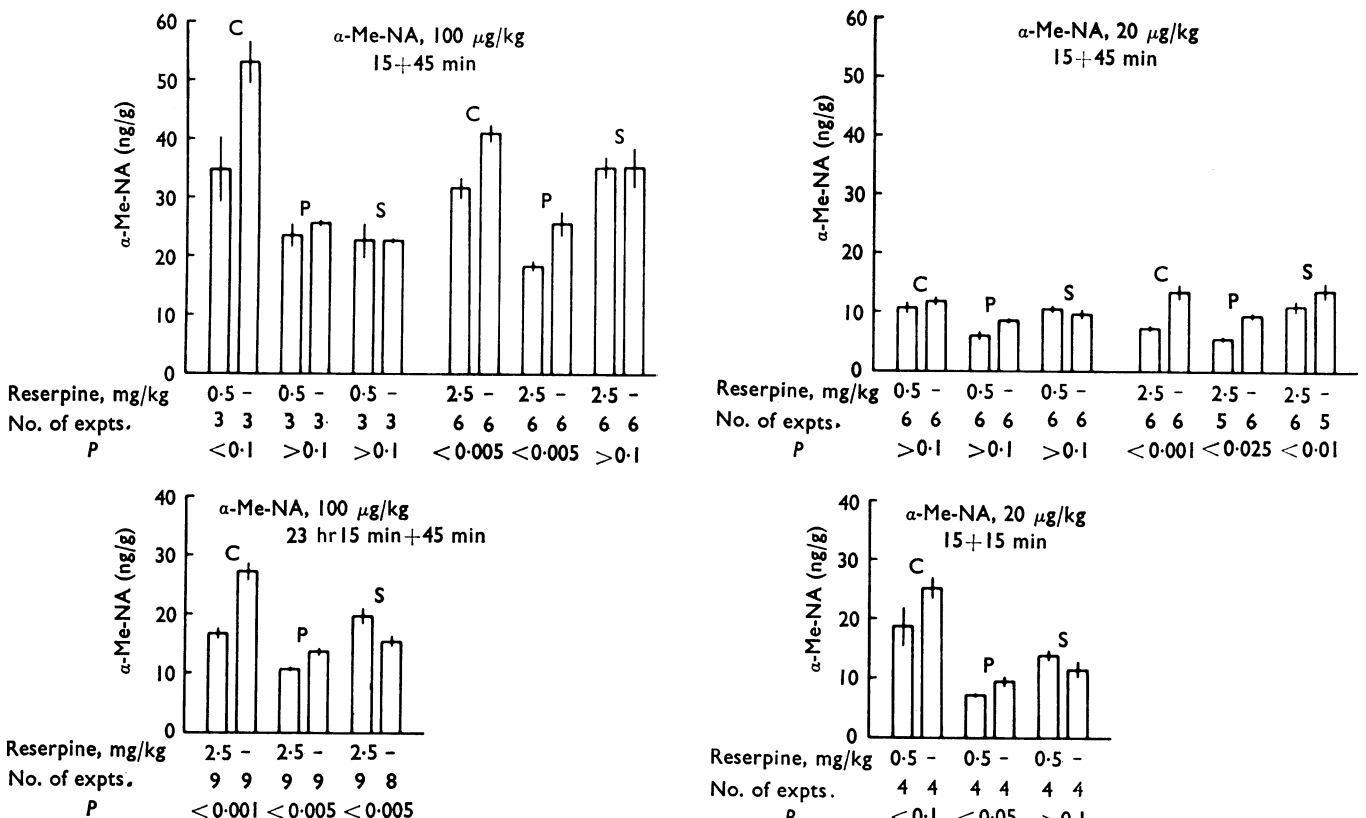

FIG. 1. Effect of reserpine on the content of previously administered ^{3}H-labelled α-methylnoradrenaline (α-ME-NA) in subcellular fractions (C, coarse fraction, P, particulate fraction, S, supernatant fraction) of the mouse heart. Vertical bars indicate standard errors of the mean. The first time indicated is the interval between injection of α-ME-NA and of reserpine; the second time is between the injection of reserpine and death.

Animals which had been given ^{3}H-α-MeNA 24 hr previously showed a loss of tritiated amine from the coarse ($P<0.001$) and particulate ($P<0.005$) fractions 45 min after intravenous injection of reserpine (2·5 mg/kg). The supernatant fraction showed a significant increase ($P<0.005$).

Effect of reserpine on the subcellular distribution of ^{3}H-α-MeNA

When the amount of ^{3}H-α-MeNA in the particulate fraction was expressed as a percentage of ^{3}H-α-MeNA in the particulate + supernatant fractions (P/(P+S) ratio) reserpine caused a pronounced decrease in most experiments (Table 1). When the larger dose of ^{3}H-α-MeNA and smaller dose of reserpine were used, P/(P+S) was unchanged; when the lower dose of α-MeNA and higher dose of reserpine were used, the decrease in P/(P+S) was not significant.

Effect of reserpine on the total content of ^{3}H-α-MeNA in the mouse heart

When reserpine was given 24 hr after administration of the labelled amine and 45 min before death, it caused a significant ($P<0.025$) decrease in the amount of ^{3}H-α-MeNA in the heart (Table 1).

Sixty minutes after administration of ^{3}H-α-MeNA (20 μg/kg) and 45 min after injection of reserpine (2·5 mg/kg), there was a decrease in the ^{3}H-α-MeNA content of the heart. There was also a decrease ($P<0.1$) when the higher dose of ^{3}H-α-MeNA and lower dose of reserpine were used.

In all other experiments reserpine did not reduce the total content of ^{3}H-α-MeNA of the heart.

TABLE 1. *Effect of reserpine on the content and subcellular distribution of ^{3}H-μ-MeNA in the mouse heart*

^{3}H-α-Me-NA dose used (μg/kg)	Reserpine dose used (mg/kg)	No. of expts.	Interval	^{3}H-α-Me-NA ± S.E. of mean (ng/g)	Significance test (*P*)	$\frac{P}{P+S\times 100}$ ± S.E. of mean	Significance test (*P*)
20	0·5	4	30 min	39·4±4·46	>0·1	34·0±1·00	<0·005
20	—	4	30 min	45·8±3·27		45·6±1·43	
100	0·5	3	60 min	80·9±8·00	<0·1	51·2±1·58	>0·1
100	—	3	60 min	101·0±3·18		53·5±0·79	
100	2·5	6	60 min	84·8±5·92	>0·1	34·5±2·28	<0·025
100	—	6	60 min	101·4±5·69		42·2±1·47	
20	0·5	6	60 min	27·0±2·08	>0·1	35·8±1·73	<0·001
20	—	6	60 min	29·7±0·70		46·7±0·30	
20	2·5	5	60 min	24·4±1·89	<0·005	32·5±2·00	>0·1
20	—	5	60 min	36·8±3·59		40·6±3·50	
100	2·5	9	24 hr	46·8±2·46	<0·025	35·4±1·41	<0·001
100	—	8	24 hr	55·1±2·97		47·4±1·08	

The animals were killed 30 min, 60 min or 24 hr after intravenous administration of ^{3}H-α-methylnoradrenaline. Reserpine was injected 15 min (30 min values) or 45 min before death. P/(P+S)×100 expresses the amount of ^{3}H-α-MeNA in the particulate fraction as a percentage of ^{3}H-α-MeNA in the particulate + supernatant fractions.

Discussion

The existence of two different mechanisms for accumulation of amines within sympathetic nerves, namely active transport through the nerve cell membrane and incorporation into the storage granules complex, is now generally accepted (Hillarp & Malmfors, 1964; Carlsson & Waldeck, 1965; Giachetti & Shore, 1966). These two mechanisms can be selectively blocked by drugs: reserpine blocks the granular uptake mechanism whereas protriptyline and desipramine block the uptake through the nerve cell membrane. After reserpine administration, the *in vivo* uptake of ^{3}H-NA as well as of ^{3}H-α-MeNA into subcellular particles of the mouse heart is considerably depressed (Stitzel & Lundborg, 1967; Carlsson *et al.*, 1967). As the cell membrane pump is not blocked by reserpine, there is an accumulation of ^{3}H-α-MeNA and, if the monoamine oxidase is inhibited, of ^{3}H-NA in the cytoplasm outside the granules. Reserpine is an effective releasing agent. It was early shown that reserpine causes release of catecholamines from sympathetically innervated organs (Bertler, Carlsson & Rosengren, 1956; Muscholl & Vogt, 1958). This release of monoamines—or block of re-uptake of the amine leaking out from the granules—is largely an intracellular event, oxidative deamination taking place before the amine released from the store escapes from the cell (Carlsson, Rosengren, Bertler & Nilsson, 1957).

In a previous study it was shown that reserpine releases previously administered ^{3}H-metaraminol almost exclusively from the particulate fraction (Lundborg & Stitzel, 1967a); however, metaraminol is incorporated into the amine-storing granules by a mechanism partly different from that responsible for the uptake of NA and α-MeNA (Lundborg & Stitzel, 1967b). Exogenously administered α-MeNA or α-MeNA formed enzymatically *in vivo* from α-methyl-dopa (Carlsson & Lindqvist, 1962; Philippu & Schümann, 1965; Lindmar & Muscholl, 1965; Schümann, Grobecker & Schmidt, 1965) can accumulate in sympathetically innervated organs and displace endogenous noradrenaline. That α-MeNA is located primarily within the adrenergic nerves is supported by the following observations: (1) denervation (Carlsson & Waldeck, personal communication) or pretreatment with protriptyline, a potent membrane pump blocking agent, prevents uptake of the amine (Carlsson *et al.*, 1967); (2) it has been shown by histochemical techniques that α-MeNA accumulates and is retained in adrenergic nerves (Malmfors, 1965); (3) moreover, α-MeNA can be recovered in particulate fractions from heart homogenates (Grobecker & Schümann, 1966; Lundborg & Stitzel, 1967b), and is released by sympathetic nerve stimulation (Muscholl & Maitre, 1963; Conradi, Gaffney, Fink & Vangrow, 1965).

In the present experiments, the values obtained from the coarse fraction are difficult to evaluate because this fraction contains nuclei, unbroken cells, membrane fragments, co-precipitated granules etc.

Reserpine usually caused a decrease in the ^{3}H-α-MeNA content of the particulate fraction except when the higher dose of ^{3}H-α-MeNA and the lower dose of reserpine were used. This decrease in the ^{3}H-α-MeNA content was most pronounced (41%) when the lower dose of ^{3}H-α-MeNA was followed by the higher dose of reserpine. Thus there seems to be competition between ^{3}H-α-MeNA and reserpine for granular binding sites.

When the higher dose of ^{3}H-α-MeNA and lower dose of reserpine were used there was no decrease in the ^{3}H-α-MeNA contents of either the particulate or the super-

natant fraction and therefore no change in the P/(P+S) ratio. When the lower dose of ^{3}H-α-MeNA and the higher dose of reserpine were used, there was a decrease in the particulate as well as in the supernatant fraction but the decrease in P/(P+S) from 32·5% to 40·6% was not statistically significant. In all other experiments there was a pronounced decrease in the P/(P+S) ratio, which was due to a releasing effect of reserpine on the granules.

No consistent results were obtained with the supernatant fraction since part of the ^{3}H-α-MeNA content of this fraction is due to release from the particulate fraction (Lundborg, 1967). The changes in the total content of ^{3}H-α-MeNA in the heart were also not consistent. Since α-MeNA is resistant to monoamine oxidase it can accumulate in the cytoplasm when it is released from the granules. This accumulation probably induces a leakage through the cell membrane.

When administered 15 min after the injection of ^{3}H-α-MeNA and 15 min before death, reserpine caused a decrease of the amine in the particulate but not in the supernatant fraction. In a similar experiment, when ^{3}H-metaraminol was used instead of ^{3}H-α-MeNA, reserpine did not change the ^{3}H-metaraminol content of any fraction (Lundborg & Stitzel, 1967a). The probable explanation of this observation is that metaraminol is first taken up by a reserpine-resistant granular mechanism and only later transferred slowly to a reserpine-sensitive storage site. Since α-MeNA, like NA, appears to have a much higher affinity for a reserpine-sensitive uptake mechanism in the granules (Lundborg & Stitzel, 1967b), these amines are almost immediately taken up into a storage site from which they are easily released by reserpine.

The results of the present experiments do not support the theory that reserpine acts by inhibition of release of amines from nerve granules.

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