observed that, in two steroid series, the acetate of 3β, 5β-axial configuration was more polar than that of its 3α, 5α-axial isomer.

We used micro-tetrazolium blue reaction for quantitative values, and obtained 200–250 μg/24 h for allo-tetrahydro 17α-hydroxy-cortexone and 100–150 μg/24 h for allo-tetrahydro-cortexone, in the subjects studied.

Discussion. The hormone of the adrenal cortex, 17α, 21-dihydroxy-pregn-4-en 3, 20-dione (Compound S), is metabolized into different hydrogenated derivatives which have been isolated from human urine. These are: 3α-17α, 21-trihydroxy-5β-pregnane 20-one (tetrahydro-17α-hydroxy-cortexone-THS)[15]; 3α, 17α, 20ξ, 21-tetrahydroxy-5β-pregnane (hexahydro-17α-hydroxy-cortexone)[16]; 17α, 21-di-hydroxy-5β-pregnane 3, 20-dione (dihydro-17α-hydroxy-cortexone)[17]; and 6β, 17α, 21-trihydroxy-pregn-4-en 3, 20-dione (6β, 17α-dihydroxy-cortexone)[18]. The allo-tetrahydro-17α-hydroxy-cortexone identified in this work, is a new metabolite of 17α-hydroxy-cortexone. It is to be pointed out that 17α, 21-dihydroxy-pregnenolone (3β, 17α, 21-trihydroxy-pregn-5-en 20-one) can also be converted *in vivo* into allo-tetrahydro-17α-hydroxy-cortexone, as we have recently shown[19].

The hormone cortexone, 21-hydroxy-pregn-4-en 3, 20-dione, is metabolized into 3α, 21-dihydroxy-5β-pregnane 20-one (tetrahydroxy-cortexone)[16] and 21-hydroxy-5β-pregnane 3, 20-dione (dihydrocortexone)[17]. The 3α, 21-dihydroxy-5α-pregnane 20-one (allo-tetrahydro-cortexone), identified in this work, is a new metabolite of this hormone.

Résumé. Le 3α, 17α, 21-trihydroxy 5α-pregnane (allo-tétrahydro-17α-hydroxy-cortexone) et le 3α, 21-dihydroxy 5α-prégnane (allo-tétrahydro-cortexone) ont été identifiés dans l'urine humaine. Ces composés avaient les mêmes migrations chromatographiques avant ou après acétylation ou oxydation que les respectifs stéroïdes de synthèse.

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Release by Sympathetic Stimulation of α-Methylnoradrenaline Stored in the Heart after Administration of α-Methyldopa

α-Methyldopa causes a long-lasting depletion of heart and brain noradrenaline[1–3]. CARLSSON and LINDQVIST found that α-methyldopamine and α-methylnoradrenaline accumulated in the brain after administration of α-methyldopa. They expressed the view that the decarboxylation products of α-methyldopa may possibly take over the functions of the physiological amines. This hypothesis has gained some support from the observation that transmission over sympathetic pathways is not inhibited in dogs whose noradrenaline stores have been depleted by 56% following α-methyldopa[4]. On the other hand, this loss of noradrenaline may have been insufficient to cause functional failure.

It was demonstrated recently that after administration of α-methyldopa the noradrenaline of the heart is partially replaced by an equipressor amount of α-methylnoradrenaline[5]. In order to find out whether sympathetic stimulation releases α-methylnoradrenaline concomitantly with noradrenaline, we have done the following experiments.

Rabbits were given four intravenous doses of 100 mg/kg of dl-α-methyldopa in the course of two days. 16 h after the last injection, the heart with the sympathetic nervous supply was isolated and perfused[6]. Untreated animals served as controls. The perfusion fluid was collected before, during and after sympathetic stimulation or during an infusion of dimethylphenyl-piperazinium iodide (DMPP). The catecholamines were adsorbed on alumina. After termination of the perfusion experiment, the right ventricle was homogenized in trichloroacetic acid and the catecholamines were adsorbed on alumina[7]. The pressor activity of the alumina eluates was assayed against standard doses of l-noradrenaline and l-α-methylnoradrenaline on the pithed rat. In this test preparation, the ratio of equipressor doses of l-α-methylnoradrenaline/l-noradrenaline (expressed as bases) was 0.95 (0.86–1.03). In aliquots of the alumina eluates, the concentrations of noradrenaline and adrenaline were estimated by differential fluorometry[8]. α-Methylnoradrenaline exhibited only 3.8% of the fluorescence of noradrenaline and less than 1% of that of adrenaline. Differential estimation of noradrenaline and α-methylnoradrenaline could therefore be performed by using the biological and fluorometric assay procedures. In control experiments, when there was no α-methylnoradrenaline in heart extracts or perfusates obtained after sympathetic stimulation or DMPP, the concentration of noradrenaline found biologically agreed well with the result of the chemical estimation (Figure). If, however, the myocardium or the perfusates were obtained from rabbits pretreated with α-methyldopa, the % ratio, fluorometric activity/pressor activity, fell significantly to less than 50% (Figure).

The noradrenaline concentration in the right ventricle of the perfused hearts of untreated rabbits was 1.39 ± 0.12 μg/g as estimated on the blood pressure, and 1.40 ± 0.09

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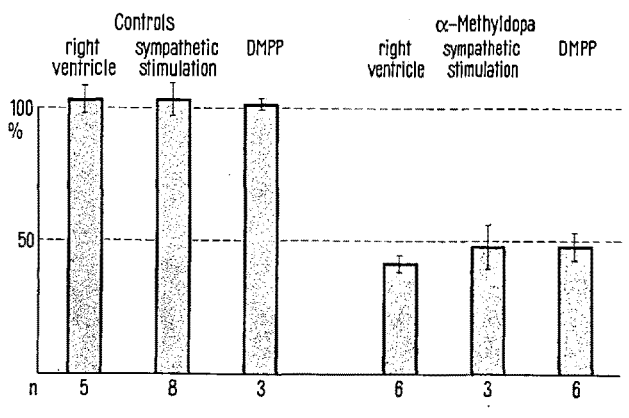
μg/g as estimated by fluorometry. These values are lower than the mean noradrenaline concentration of 1.98 μg/g which was found when the right ventricle was worked up immediately after death[9]. The lower concentration in the perfused heart might be due to an increase of intracellular fluid and/or to partial exhaustion of the transmitter by the stimulation procedures.

After the four doses of α-methyldopa, the noradrenaline concentration of the heart was reduced by 41% to 0.82 ± 0.05 μg/g. However, the myocardium had accumulated a concentration of α-methylnoradrenaline of 1.63 ± 0.11 μg/g, as calculated according to the differential assay.

In a number of experiments the sympathetic supply of the heart was stimulated for 30 sec with rectangular pulses of 10/sec followed by a resting period of 30 sec. This schedule was repeated for 6–9 min. The perfusates of the control hearts contained 116 ± 17 ng noradrenaline/min stimulation. Biological and fluorometric assays gave the same results (Figure). Sympathetic stimulation of three hearts of rabbits pretreated with α-methyldopa caused an increase in rate and force of contraction indistinguishable from that produced in control hearts. The mean amount of pressor amines released into the perfusate was 92 ng/min of stimulation (expressed as noradrenaline). The range (36–172 ng/min) is well covered by the range found in a large series of experiments performed on control hearts[6]. After stimulation the ratio fluorometric activity/pressor activity in the perfusates was not significantly different from the ratio observed in the myocardium (Figure). This indicates that α-methylnoradrenaline is released by sympathetic stimulation in the same proportion to noradrenaline as it is found in the total amine store.

When the hearts of pretreated animals were infused with DMPP at a final concentration of 9-21 μg/ml, there was an increase in rate and force of contraction similar to the effects seen in normal hearts. The mean quantity of pressor amines released by DMPP (expressed as noradrenaline) was 182 ng/min (range 107–320). The individual values fell within the scatter of values representing the relation between dose of DMPP and noradrenaline release in control hearts as shown in Figure 2 of a previous paper[10]. Following pretreatment with α-methyldopa the ratio fluorometric activity/pressor activity after DMPP was similar to the ratios found in the myocardium and in the perfusates collected during sympathetic stimulation.



Effect of α-methyldopa on the ratio noradrenaline/total vasopressor catecholamines in the right ventricle and in perfusates after sympathetic stimulation of the isolated heart and after infusion of DMPP. The columns represent means (± s.e.) of the noradrenaline estimated fluorometrically as % of the pressor activity assayed on the pithed rat (and expressed as noradrenaline). n: number of estimations. α-Methyldopa: rabbits received 4 × 100 mg/kg i.v. in 2 days. Hearts perfused 16 h after the last dose. DMPP: 9–21 μg/ml.

In the myocardium of animals pretreated with α-methyldopa, adrenaline was present as 1.4% of the noradrenaline concentration. This low value did not differ from the percentage of adrenaline found in hearts of normal rabbits[9] and in perfusates after sympathetic stimulation[6] or DMPP[10].

The presence of α-methylnoradrenaline in perfusates from hearts of α-methyldopa-pretreated rabbits after sympathetic stimulation or infusion of DMPP was also demonstrated by paper chromatography. Phenol-HCl was used[11] in order to separate α-methylnoradrenaline from noradrenaline. The eluates of the paper strips were assayed on the blood pressure, and the same proportion between the amounts of noradrenaline and α-methylnoradrenaline was found as calculated from the differential estimations. α-Methyldopamine has a higher Rf-value than noradrenaline and α-methylnoradrenaline in the chromatogram and could not interfere with the biological estimation because its pressor activity was only 1.1% of that of noradrenaline and α-methylnoradrenaline, respectively.

In doses up to 1 mg, α-methyldopa had no pressor activity (less than that of 1 ng noradrenaline) and therefore did not interfere with the biological assay. Since α-methyldopa caused 35% of the fluorescence produced by noradrenaline, its presence could have interfered with the chemical estimation of noradrenaline. However, the possibility was excluded that α-methyldopa was still retained by the hearts 16 h after the last injection and was leaking into the perfusion fluid, since the perfusates collected before and after sympathetic stimulation did not contain substances giving the fluorescence reaction.

It can be concluded that α-methylnoradrenaline which is formed after administration of α-methyldopa is at least partly stored in the heart and is released from it by sympathetic stimulation or by DMPP. The sensitivity of the adrenergic receptor involved determines whether α-methylnoradrenaline behaves like a 'false' or like the physiological transmitter.

Résumé. Dans les cœurs de lapins traités par l'α-méthyldopa, la déplétion de la noradrénaline s'accompagne d'une fixation importante d'α-méthylnoradrénaline. Sous l'effet de stimulation sympathique ou d'iodure de diméthylphényl-pipérazinium, ces cœurs libèrent conjointement de la noradrénaline et de l'α-méthylnoradrénaline.

Les quantités respectives de noradrénaline et d'α-méthylnoradrénaline libérées par stimulation sympathique sont les mêmes que celles libérées par l'iodure de diméthylphényl-pipérazinium et que celles trouvées dans le myocarde lui-même.

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