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Inhibitory effect of two steroids on the accumulation of [^{3}H]metaraminol by rat tissues in vivo*

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The present experiments were designed to investigate the effect of methylprednisolone and dexamethasone on tritiated metaraminol uptake in the whole animal. Rats received i.p. injections of methylprednisolone (5, 10 and 20 mg kg^{-1}) or dexamethasone (1, 2 and 4 mg kg^{-1}) on each of 3 consecutive days. Under light ether anaesthesia 5 min before death the rats received radioactive metaraminol (3 μg kg^{-1}) via the femoral vein. Samples of various organs were removed and prepared for scintillation counting. The highest radioactivity was observed in the lungs and heart, followed by the kidney medulla, aorta, kidney cortex and submaxillary gland. Both steroids significantly inhibited uptake of metaraminol in the tissues examined. Dexamethasone seems to be more potent than methylprednisolone. It is concluded that these steroids may modify the uptake mechanism responsible for inactivation of the sympathomimetic neurotransmitter.

We have previously described the uptake and release of [^{3}H]metaraminol in different tissues in vitro and in vivo (Davila & Khairallah 1970; Khairallah et al 1971; Davila & Davila 1976). We have also demonstrated that various substances, e.g. angiotensin II, ouabain, tyramine, markedly inhibited the uptake of this sympathomimetic drug (Khairallah et al 1971; Davila & Davila 1975). Others have described a similar action of steroids on catecholamine uptake and release in tissues and organs in vitro (Iversen & Salt 1970; Almgren & Jonason 1973; Iwasawa & Gillis 1973) or in vivo (Almgren & Jonason 1973).

The effect of methylprednisolone and dexamethasone on [^{3}H]metaraminol accumulation in various organs has therefore been investigated in the intact rat. The present experiments suggest that steroid pretreatment reduces this accumulation.

MATERIALS AND METHODS

Female Wistar rats, 150–200 g received three daily intraperitoneal doses of methylprednisolone (5, 10 and 20 mg kg^{-1}) or dexamethasone (1, 2 and 4 mg kg^{-1}) and were killed 24 h after last injection. Control animals received the appropriate vehicle.

Methylprednisolone was administered as the sodium salt of 21-hemisuccinyl-6α-methylprednisolone (Urbason-Solubile, Hoechst AG, Frankfurt am Main, West Germany), and dexamethasone as the disodium salt of dexamethasone-21-phosphate (Dexamethason, Lek, Ljubljana, Yugoslavia). All doses refer to parent steroid. Under light ether anaesthesia 5 min before death the rats were given 3 μg kg^{-1} (±)-metaraminol- [7-^{3}H (N)], (New England Nuclear Corp., Boston, Mass., USA); specific activity 6·72 Ci mmol^{-1} (249 GBq mmol^{-1}) via the femoral vein. Rats were killed by cervical dislocation before the cannulation of the abdominal aorta for the collection of blood samples. In the present experiments the plasma time level curve of [^{3}H]metaraminol was not followed. The time of killing the rats 5 min after application of [^{3}H]-metaraminol was chosen from preliminary experiments. The organs were removed and tissue samples rinsed three times in ice-cold distilled water, blotted on filter paper and weighed. Tissues were solubilized in Protosol (New England Nuclear Corp.) and a scintillation solution (0·4% PPO, 0·01% POPOP in distilled toluene) was added. Samples in duplicate were counted for 20 min three times in a Packard TRI-CARB spectrometer with counting efficiency ranged from 20–25%. The total radioactivity counted was assumed to represent unchanged metaraminol since metaraminol is not a substrate for either monoamine oxidase or catechol-*O*-methyltransferase (Giachetti & Shore 1966).

Dunnett's (1955) two-sided *t*-test was used to evaluate statistical data. A probability level of less than 0·05 was used as the criterion of significance. The dose of steroid causing 50% change in uptake was also determined.

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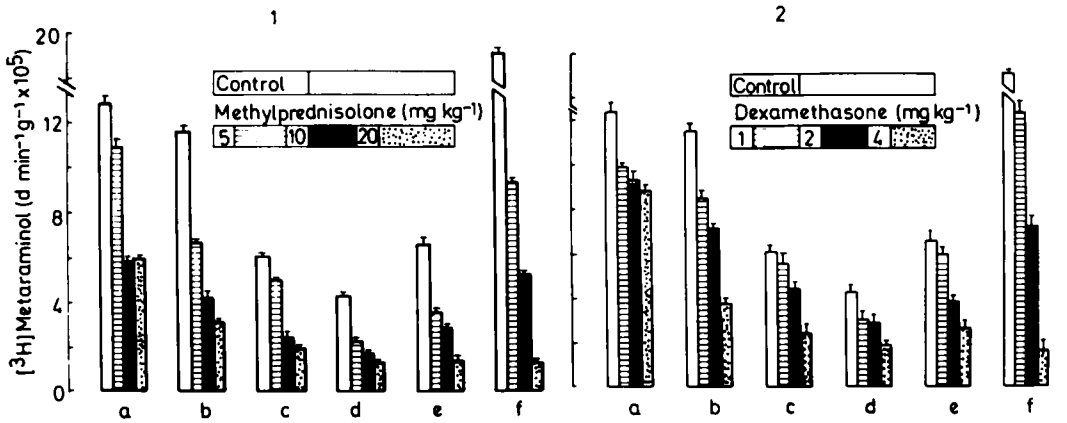

FIG. 1. Uptake of [³H]metaraminol after 3-days i.p. administration of methylprednisolone in auricles (a), left ventricle (b), aorta (c), kidney cortex (d), kidney medulla (e) and lungs (f). Each bar represents the mean ± s.e.m. of 5–6 determinations.

FIG. 2. Uptake of [³H]metaraminol after 3-days i.p. administration of dexamethasone in auricles (a), left ventricle (b), aorta (c), kidney cortex (d), kidney medulla (e) and lungs (f). Each bar represents the mean ± s.e.m. of 5–6 determinations

RESULTS

Concentrations of tritium were highest in the lungs and heart, followed in decreasing order by kidney medulla, aorta, kidney cortex and submaxillary gland. Both steroids caused a dose-related fall in the [³H]metaraminol content in the tissues examined. As shown in Fig. 1 methylprednisolone caused the most pronounced inhibition of [³H]metaraminol uptake in lung and kidney medulla. In these tissues the highest dose of methylprednisolone elicited 92 and 79% inhibition, respectively.

Pretreatment of rats with dexamethasone also greatly reduced the accumulation of [³H]metaraminol in tissues examined. The greatest inhibition was obtained in left ventricle, aorta and lungs (Fig. 2).

The effect of both steroids upon the radioactivity in rat submaxillary gland is presented in Table 1. Here the uptake was also markedly reduced.

When [³H]metaraminol radioactivity was determined in the serum of steroid-treated rats there was a dose-related increase of radioactivity (Fig. 3).

Table 2 shows doses of methylprednisolone and dexamethasone to cause 50% inhibition of [³H]-metaraminol uptake in various organs.

Table 1. The effect of methylprednisolone and dexamethasone on the accumulation of [³H]metaraminol by rat submaxillary gland. Steroids were given i.p. for 3 consecutive days (n = no of rats).* Mean ± s.e.m.

Dose (mg kg⁻¹)	[³H]metaraminol (d min⁻¹ g⁻¹ × 10⁵)	n	Inhibition (%)	P
control	1·9 ± 0·2*	6	0	—
Methyl-prednisolone				
5	1·6 ± 0·1	5	16	>0·05
10	1·5 ± 0·1	6	21	>0·05
20	0·7 ± 0·2	6	65	<0·01
Dexamethasone				
1	1·6 ± 0·2	6	16	>0·05
2	1·3 ± 0·2	5	32	>0·05
4	0·8 ± 0·1	6	58	<0·01

DISCUSSION

The present study indicates that after 3-days treatment of rats with methylprednisolone or dexamethasone there is a dose-related decrease of [³H]metaraminol accumulation in the tissues examined. The most striking effect was observed in the lung, an organ

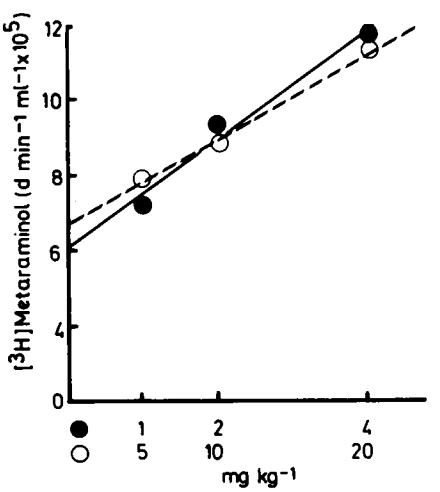

FIG. 3. Concentration of [³H]metaraminol in serum. Effect of 3-days i.p. administration of methylprednisolone (○, r = 0·999; y = 0·2x + 4·7) and dexamethasone (●, r = 0·994; y = 1·5x + 4·0). Each point represents the mean of 5–6 determinations.

Table 2. Doses (mg kg^{-1}) of methylprednisolone I and dexamethasone II causing 50% inhibition of [^{3}H]metaraminol accumulation. Data derived from the results of Figs 1, 2 and Table 1.

Tissue	I	II
Auricle	16·1	11·3
Left ventricle	9·8	2·7
Aorta	10·7	3·4
Kidney cortex	7·6	3·4
Kidney medulla	6·8	3·1
Lungs	3·6	1·6
Submaxillary gland	16·7	3·4

which showed the highest uptake of [^{3}H]metaraminol. A similar inhibition of uptake of noradrenaline and metaraminol has been observed by others. For example, in the experiments with isolated perfused rabbit lungs cortisone, hydrocortisone and corticosterone caused inhibition of noradrenaline uptake by 14, 16 and 22%, respectively (Iwasawa & Gillis 1973). Furthermore, the inhibition of noradrenaline or 5-hydroxytryptamine uptake in similarly perfused rabbit lungs was obtained with a variety of drugs, e.g. cocaine, phenoxybenzamine, phentolamine, imipramine (Iwasawa & Gillis 1974). Similar observations have been reported by Davila & Davila (1975, 1976, 1978) on incubated lung tissue. These authors clearly demonstrated the existence of extraneuronal transport for metaraminol in the lungs. Our present results support the concept of Iversen & Salt (1970) that steroids act on $Uptake_2$.

The observed reduction in [^{3}H]metaraminol accumulation by heart due to pretreatment with steroids may explain the potentiated response of this organ to sympathetic stimulation reported by Kaumann (1972). He observed that hydrocortisone elicited an increase of cardiac action induced by noradrenaline or isoprenaline. Using rat isolated hearts Iversen & Salt (1970) showed that oestradiol, corticosterone, deoxycorticosterone and progesterone inhibited the uptake of [^{3}H]metaraminol in a dose-related manner.

Since in our present experiments a similar inhibition of [^{3}H]metaraminol uptake was achieved in aorta, this may be connected with the augmented response of vascular smooth muscle described by Zweifach et al (1953) and Kalsner (1969).

Results obtained with kidney cortex and kidney medulla reflect the ability of steroids to inhibit uptake of [^{3}H]metaraminol in those tissues with good sympathetic innervation, and these findings are consistent with the view that the amine uptake mechanism is the main process involved in the inactivation of the neurotransmitters proposed by Iversen et al (1966) and Hughes (1972).

In the experiments of Almgren & Jonason (1973) an enhanced secretory response of rat submaxillary gland to noradrenaline was obtained following the application of corticosterone. This effect was attributed to the inhibition of noradrenaline uptake. In our present experiments both steroids inhibited very significantly uptake ($r = 0.998$ for methylprednisolone and $r = 0.972$ for dexamethasone, $P<0.01$) of [^{3}H]metaraminol in the rat submaxillary gland.

With increasing doses of methylprednisolone and dexamethasone there is an increase of [^{3}H]metaraminol concentration in serum. This most probably reflects the inhibitory action of these steroids on [^{3}H]metaraminol accumulation in tissues thus causing it to remain in the serum.

On comparison of doses causing 50% inhibition of [^{3}H]metaraminol accumulation it appears that dexamethasone is more potent than methylprednisolone.

Thus our present data, together with observations of other authors, clearly demonstrate the inhibitory effect of steroids on [^{3}H]metaraminol uptake in vivo by various organs in rats. This may be of importance when steroids are used with sympathomimetics.

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