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Effect of dopamine and long-chain *N*-acylethanolamines on steroidogenesis in rat adrenal gland *in vitro*

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Abstract: Recent experiments in several laboratories have shown that *N*-acylethanolamines (NAEs) modulate some membrane functions. In the adrenal cortex, the membranes are the structures responsible for steroidogenesis. We investigated the effect of long-chain NAEs with saturated and unsaturated acyls on dopaminergic control of adrenal steroidogenesis. In the presence of dopamine (10^{-6} M) the output of 11-hydroxycorticosteroids and aldosterone labelling from ^{3}H-cholesterol increased by 20%. Simultaneous addition of *N*-stearoylethanolamine and dopamine had no effect on steroid output. Upon joint addition of dopamine and a mixture of NAEs with unsaturated acyl residues, 11-hydroxycorticosteroid output rose by 33%.
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Introduction: Increasing interest was focused on *N*-acylethanolamines (NAEs) and their effect on membranes and lipid bilayers. NAEs also affect ion transport, lipid peroxidation and many other physiological and biochemical processes (see [1,2]). One of the NAEs, *N*-arachidonoylethanolamine (anandamide), is the endogenous compound that specifically binds to brain-type cannabinoid receptors and which is the natural ligand for these receptors [2,3]. Injected intraperitoneally into rats, it changed their motor behaviour and reduced the content of dopamine as well as inhibiting the activity of a key enzyme of dopamine synthesis, tyrosine hydroxylase, in the corpus striatum. Simultaneously it lowered the ratio of D1/D2 dopamine receptors in this nucleaus [3].

Recently it has become evident that other long-chain NAEs with saturated and unsaturated acyl residues can realize some effects which were described for anandamide. Cannabinoid receptors have been detected in many organs and tissues, particularly in human adrenal gland [4]. However, their function in this gland is unkown.

In the adrenal cortex, dopamine seems to exert the tonic inhibitory effect on aldosterone secretion (see [5–7]. The influence of dopamine on glucocorticoid production has not been sufficiently studied. Dopamine induced short-term activation of aldosterone and corticosterone secretion by perifused dispersed frog interrenal cells, followed by long-term inhibition of hormone secretion [8].

The effect of dopamine on cAMP production depends upon the receptor type [9]. The dopamine receptors of D1 and D2 subtypes were found in adrenal gland tissues [9]. However, Amenta *et al.* [10] using radioligand binding techniques, could not find receptors of the D1 subtype in human adrenal glands. The D2 subtype dopamine receptors were localized mainly in the zona glomerulosa, where aldosterone is synthesized. Very recently the mRNA for peripheral D_{1A} receptors was found and the presence of these receptors in the zona glomerulosa of adrenal cortex confirmed [11]. Until now the possibility of modulations of glucocorticoid production by NAEs has remained unstudied. The possibility of relations between the regulatory effects of dopamine and NAE is unknown.

The aim of this work was to analyse the effect of dopamine and long-chain NAEs with saturated and unsaturated acyls on steroidogenesis in slices of rat adrenals.

Materials and methods: Female Wistar rats weighing 150–200 g were obtained from IEM, Kyiv, Ukraine. They were housed at room temperature and natural light conditions with food and water available *ad libitum*. Animals were lightly anaesthetized with ether and killed by decapitation.

Slices of adrenocortical tissue 0.5 mm thick were prepared on ice and transfered to tubes. Each sample contained about 10 mg of tissue. The slices were rinsed by ice-cold medium contained lactalbumine (0.5%) in Hanks solution (Plant of Bacterial Products, Moscow, Russia). After this the fresh portion of the same medium with 10 mM HEPES (pH 7.4) (Calbiochem, Luzern, Switzerland) were added to each tube. Dopamine (Merck, Darmstadt, Germany) and *N*-stearoylethanolamine (final concetration 10^{-5}–10^{-7} M) were added to the samples.

The mixture of NAEs was synthesized from plant oil in the laboratory of Dr V.E. Vaskovsky of the Institute of Marine Biology (Vladivostok, Russia). It contained unsaturated fatty acids – 18:1 ω 9; 18:2 ω 6; 18:3 ω 3; 20:1 ω 9; 22:1 ω 9 to the extent of 85%. The content of NAE 20:4 ω 6 (anandamide) in this preparation was less than 1%. The average molecular weight of the mixture of NAEs was taken as 327 and they were added to incubation media up to concentrations of 10^{-7}–10^{-5} M.

^{3}H-cholesterol (20 μKu/ml) (Izotop, St. Petersburg, Russia) was added to tubes. The samples were incubated at 37°C for 120 min with continuous shaking. The incubation was stopped by transfering the tubes to an ice-cold bath.

11-hydroxycorticosteroids were quantitatively determined

by the spectrofluorimetric method [12]. The slices were homogenized in chloroform. The chloroform extraction was repeated twice. The pooled chloroform extract was evaporated. To evaluate the incorporation of ^3H from cholesterol into corticosterone and aldosterone these steroids were separated by thin-layer two-dimensional chromatography on silica gel in the systems: chloroform – ethanol 96 : 4 (I dimension); benzene – acetone 60 : 40 (II dimension).

To visualize hormones under UV-light the cold compounds were added to the labelled ones. The radioactivity of synthesized hormones was determined in a Beckman LS 5000 liquid scintillation counter.

Student's *t*-test and the non-parametric Wilcoxon-Mann-Whitney U-test were used for statistical comparisons of the data.

Results: As shown in Figure 1, the effect of dopamine on the label incorporation into aldosterone and corticosterone depended on the dopamine concentration. Dopamine in concentrations of 10^{-7}–10^{-6} M did not change the label incorporation into corticosterone. The radioactivity of aldosterone slightly increased at a dopamine concentration of 10^{-6} M. At 10^{-5} M, the ^3H-incorporation decreased in both aldosterone and corticosterone.

Addition of *N*-stearoylethanolamine or the mixture of NAEs at concentrations of 10^{-7}–10^{-6} M did not cause significant changes of label incorporation into aldosterone or corticosterone (Figure 2a, b). However, the radioactivity of hormones increased in the presence of a 10^{-5} M NAEs mixture (Figure 2a). NAE-18:0 (10^{-5} M) had a similar effect on corticosterone labelling (Figure 2b).

To analyse possible potentiation of dopamine and NAEs effects the agonists were used at concentration of 10^{-6} M. Taken separately at this concentration the two NAEs failed to alter corticosteroids labelling. Dopamine produced a 20% increase in aldosterone labelling (Figure 1). Upon joint addition of dopamine and the mixture of NAEs the incorporation of ^3H into aldosterone and corticosterone was enhanced by 83% and 50%, respectively in comparison with basal incorporation (Figure 3). Dopamine and NAE-18:0 stimulated the incorporation of ^3H into corticosterone by approximately 80% but labelling of aldosterone changed insignificantly.

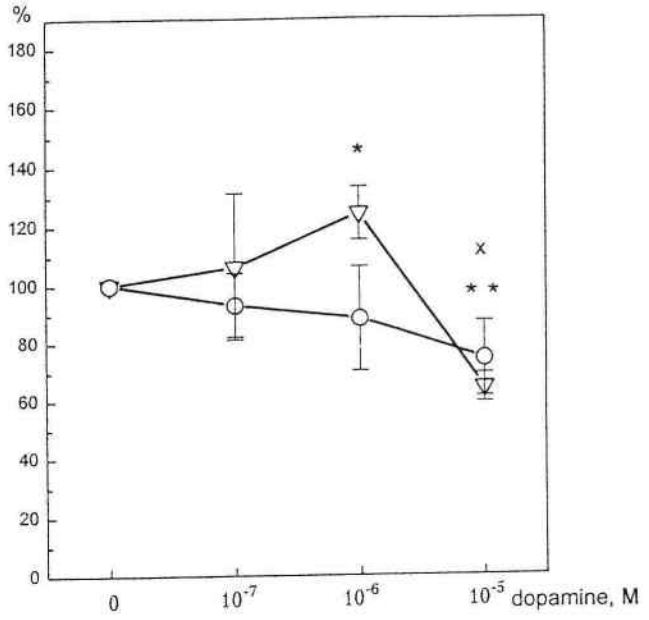
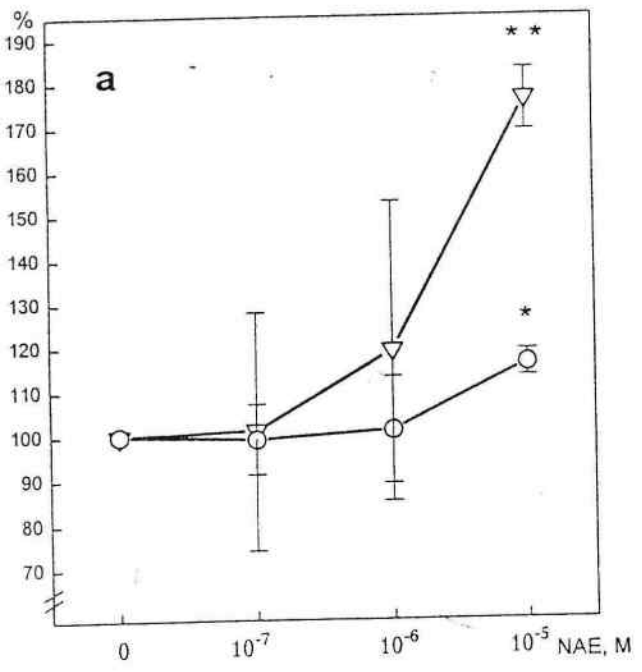


*Figure 1. Effect of dopamine on aldosterone and corticosterone labelling (means ± SEM; n = 3). Incorporation of ^3H from cholesterol into aldosterone (∇) and corticosterone (○) in rat adrenal slices. Results of three experiments are expressed in terms of percentage of control (100%). As compared with control without dopamine *P < 0.05; **P < 0.01 for aldosterone labelling (t-test); xP < 0.05 for corticosterone labelling (U-test).*



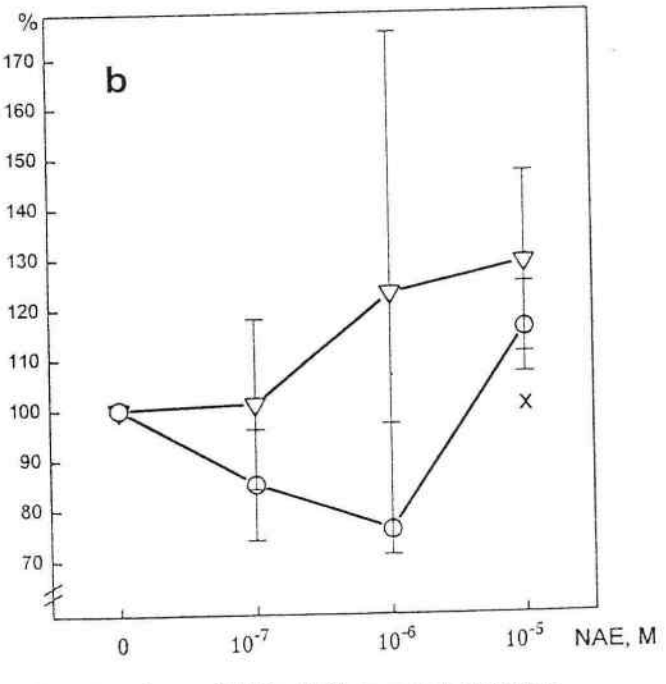


*Figure 2. Effect of NAEs on aldosterone and corticosterone labelling. (a) mixture of NAEs; (b) N-stearoylethanolamine (means ± SEM: n = 3). Incorporation of ^3H from cholesterol into aldosterone (∇) and corticosterone (○) in rat adrenal slices. Results of three experiments are expressed in terms of percentage of control (100%). As compared with control without NAEs *P < 0.05; **P < 0.01 (t-test); xP < 0.05 (U-test).*

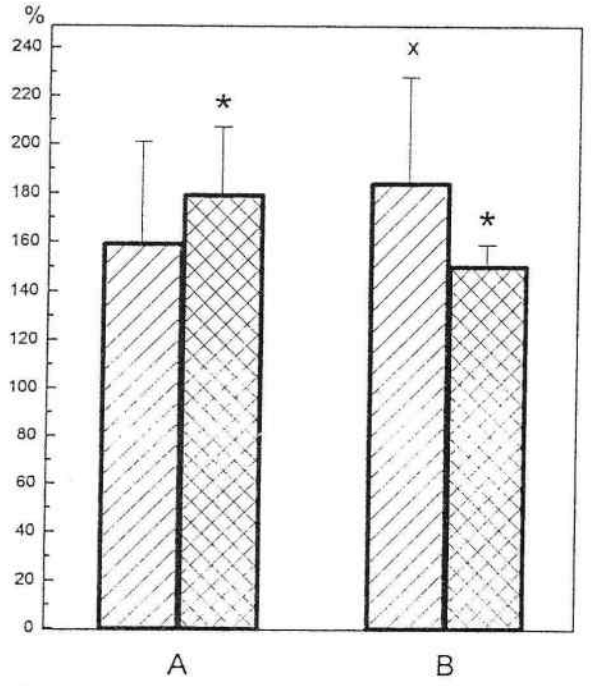


Figure 3. Effect of the joint actions of dopamine and NAEs on aldosterone (A) and corticosterone (B) labelling (means ± SEM; n = 3). Dopamine and NAEs were added at a concentration of 10^{-6} M. Hatched bars: N-stearoylethanolamine, crosshatched bars: NAEs mixture. Results of three experiments are expressed in terms of percentage of control without dopamine and NAEs. As compared with control without dopamine and NAEs $^{*}P < 0.05$ *(t-test);* $^{x}P < 0.05$ *(U-test).*

The slight activating effect of dopamine on steroidogenesis *in vitro* was confirmed by determination of the increased amounts of 11-hydroxycorticosteroids, which were represented mainly by corticosterone. In the presence of dopamine (10^{-6} M) 11-hydroxycorticosteroid production increased by 20%, from 17.68 ± 0.85 μg/g in the control to 21.55 ± 2.39 μg/g of the tissue. *N*-stearoylethanolamine caused a similar effect. Steroid output increased to 21.93 ± 1.06 μg/g of the tissue ($P < 0.01$).

Simultaneous addition of dopamine and *N*-stearoylethanolamine to the incubation mixture lead to the disappearance of their effect of 11-hydroxycorticosteroid production. The output in this case was 18.49 ± 0.96 μg/g of the tissue. Particularly under these conditions, ^{3}H-cholesterol incorporation into corticosterone increased (Figure 3). The maximal 11-hydroxycorticosteroid production (23.53 ± 1.77 μg/g of the tissue ($P < 0.01$) in comparison to the control) was determined when dopamine was added simultaneously with the mixture of unsaturated NAEs.

Discussion: The possible inhibition of aldosterone and corticosterone secretion by dopamine was confirmed in experiments *in vitro* with labelled cholesterol. However, this inhibition occurred only with a high dopamine concentration (10^{-5} M).

An earlier report [9] showed that in freshly isolated zona glomerulosa cells dopamine did not change aldosterone production. A weak inhibition of aldosterone secretion can be observed under D1 receptor blockade [9]. In cells that had been maintained in culture for three days Gallo-Payet *et al.* [9] found an increase in aldosterone synthesis and cAMP accumulation.

These conflicting results were explained by the existence of D1 and D2 types of dopamine receptors in freshly prepared cells. These two types of receptors determinated the diametrically opposed changes in cAMP level under dopamine action. During the cultivation of cells the expression of D2 subtype receptors decreased and expression of D1 subtype receptors increased. It is widely believed that the rise in cAMP is provided by D1 subtype of dopamine receptors. However, D2 subtype provides the inhibition of adenylate cyclase (see [13]). cAMP is the most significant second messenger which takes place in steroidogenesis activation [14].

The question of the dopamine effect on corticosteroid synthesis is rather complicated. Dopamine receptors are located mainly in the zona glomerulosa [6,7], but corticosterone is produced in the zona fasciculata. One can propose that the effect of dopamine on corticosterone synthesis is realized through autocrine or paracrine mediators, produced in the zona glomerulosa or in medullary chromaffin cells after dopamine binding with receptors. Dopamine in particular increased basal and stimulated release of interleukin-6 from the zona glomerulosa [15]. The effect of IL-6 on corticosteroid synthesis is not in doubt [16,17].

In our study, activation of steroidogenesis *in vitro* was determined both by the increased amount of synthesized 11-hydroxycorticosteroids and by the rise in incorporation of the label from ^{3}H-cholesterol into corticosterone. NAE-18:0 (10^{-6} M) enhanced 11-hydroxycorticosteroid production by 24% ($P < 0.02$). To increase incorporation of the label, a concentration of 10^{-5} M of NAE-18:0 was needed.

Simultaneous addition of dopamine and NAE-18:0 significantly increased the incorporation of the label into corticosterone. ^{3}H incorporation into aldosterone and accumulation of 11-hydroxycorticosteroids rose only in the presence of dopamine and the NAEs mixture, which contained mainly long-chain unsaturated acyls.

It is possible to discuss different mechanisms of NAEs action on steroidogenesis. Some NAEs can probably bind with the cannabinoid receptors in the adrenal cortex [4]. Activation of cannabinoid receptors leads to the inhibition of cAMP accumulation. G-proteins participate in this process, which is blocked by pertussis toxin [18]. However, the decrease in cAMP level would have caused the reduction of corticosteroid output, but not the increase as shown in Figure 2.

However, another explanation for the NAE effect can be proposed. In particular NAE-16:0 activated $^{86}Rb^{+}$ influx and inhibited its exflux from neuroblastoma cells, thus modulating monovalent ion transport through the plasma membrane. Some NAEs take part in regulating the function of fast sodium channels [19].

The Na^{+}/K^{+} ratio is one of the most significant regulatory factors, which determinates the rate of aldosterone synthesis and also affects glucocorticoid production (see [14]). So the changes in monovalent ion transport in adrenocortical cells caused by different NAEs may explain the discovered changes in steroidogenesis. However, it remains unclear which of the above ways is involved in the activation of adrenal function by different NAEs.

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