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Inhibition of the Renin-Angiotensin System by Pinealectomy in Female Rats

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The influence of the pineal gland on the renin-angiotensin system has been studied in female rats. Pinealectomy produces a decreased plasma renin activity (PRA) concomitant with an increased corticosterone level, and with no changes in plasma aldosterone. Administration of indol melatonin reverses the effects of pinealectomy. On the other hand ovariectomy induces an increased PRA while decreasing corticosterone and aldosterone levels. However when pinealectomy and ovariectomy are both performed, neither PRA nor corticosterone changes. These results suggest that the pineal gland may exert an influence on the renin-angiotensin system via adrenal and/or ovarian pathways.

Plasma renin activity (PRA) has been shown (7, 9, 10) to increase slightly in pinealectomized rats. This PRA increase was suggested to reflect a possible hyperactivity of the sympathetic nervous system (8).

On the other hand, the pineal gland has been reported as exerting inhibitory influences in rat adrenal glands (3, 4, 16). Furthermore, the pineal gland has been demonstrated to influence pituitary-adrenal homeostasis by both ovarian dependent and independent mechanisms (15).

If the influence of the adrenals on renin secretion by the kidneys (2, 11), is considered, there arises the possibility that the pineal may exert an influence on the renin-angiotensin system by means of its antiadrenal and/or antigonadal effects.

The present paper suggests that the renin-angiotensin system is depressed by pinealectomy in female rats by an increase in mineralocorticoid secretion, and that this effect is reversed by ovariectomy.

Materials and Methods

Female Wistar rats, weighing 150-160 g were pinealectomized and/or ovariectomized. Rats were divided in eight groups and randomly housed in 3-4 per clear plastic cages, with food and tap water available *ad libitum*. Room temperature (22 ± 0.5 °C) and lighting photoperiod (12h light : 12h dark) were controlled.

Pinealectomy was performed under Equi-Thesin anesthesia (0.5 ml/100 g) following the procedure of HOFFMAN and REITER (6), and ovariectomy, by using a conventional lumbar approach. Sham-operated animals (controls) were prepared by the same techniques with the exception that the glands were not removed. The brains of all animals were sectioned to determine the completeness of pinealectomy and the possibility of damage to surrounding brain tissue. Only animals in which pinealectomy had been complete and whose adjacent brain structures remained intact, were included in the study.

The following experimental groups were studied:

Experiment I: three groups of animals were maintained in the previously described conditions and killed at 10, 20 and 35 days after pinealectomy (P-10; P-20 and P-35 groups, respectively). Another three groups were sham-operated as controls.

Experiment II: pinealectomy and ovariectomy in two groups of rats were performed. These groups were killed at 10 and 20 days postoperatory procedure (P + 0-10 and P + 0-20 groups). As controls, two sham-pinealectomized and sham-ovariectomized groups were prepared.

Experiment III: in this experiment two groups of rats were ovariectomized and maintained during 10 and 20 days as the preceding experiments and then, killed (0-10 and 0-20 groups, respectively). Similarly, two control sham-operated groups were prepared.

Experiment IV: in order to evaluate the role of melatonin in pinealectomy-induced alterations, a group of pinealectomized rats were treated with melatonin. Melatonin (Sigma) was injected s.c. (400 μg/0.5 ml saline/day) for 10 days after pinealectomy and then, killed (P+M-10 group). Another group given 0.5 ml saline s.c./day for 10 days postpinealectomy served as control.

At the end of each experimental period, the rats were bled by aortic punction unther Equi-Thesin anesthesia between 9-10 a.m. The blood obtained was divided in two aliquots, plasma (with EDTA-Na_2) and serum, and immediately frozen to —20 °C for subsequent analysis.

Serum electrolytes were determined by flame photometry and no changes were found in any group. PRA and aldosterone were determined by radioimmunoassay (5, 14), while corticosterone was measured by acid fluorescence (12).

Statistics: all results are expressed as the mean ± S.E.M. PRA, aldosterone and corticosterone as percentages of their respective sham-operated controls.

Statistical significance of mean differences was evaluated by using Student's *t* test. Welch test was employed when heterogeneity of variance was encountered.

Results

Figure 1 summarizes the effects of pinealectomy on plasma renin activity (PRA), aldosterone and corticosterone levels (P-10, P-20 and P-35 groups). Pinealectomy significantly decreased PRA levels at 10, 20 and 35 days postoperative procedure (P-10: —30.86 ± 6.48; P-20: —26.34 ± 9.24; P-35: —32.33 ± 11.16). Aldosterone secretion was unaffected by pineal removal in all age groups.

While pinealectomy raises corticosterone levels, it disminishes, by contrast, PRA. Corticosterone is significantly en-

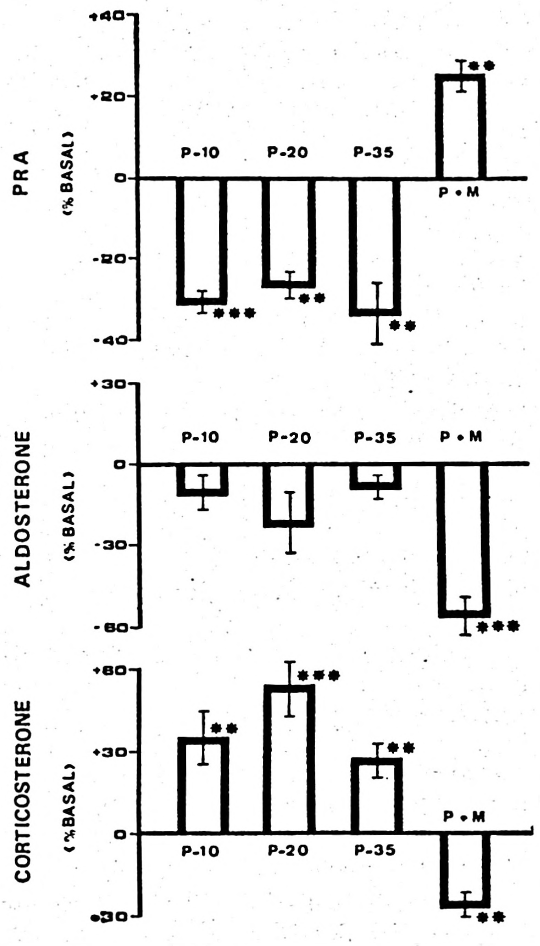

Fig. 1. *Effects of pinealectomy and melatonin treatment on percentages of PRA, aldosterone and corticosterone basal levels in 10, 20 and 35 old day groups.*
$p < 0.01$: ***; $p < 0.05$: **.

hanced in all pinealectomized groups (P-10: $+34.67 \pm 13.07$; P-20: $+53.12 \pm 11.99$; P-35: $+23.00 \pm 8.43$), coinciding with a decrease in PRA.

In melatonin treated pinealectomized rats (P+M-10 group), the pinealectomy-induced effects on these parameters are reversed.

PRA increases $+27.62 \pm 8.28$ percent as against control, while corticosterone falls -26.17 ± 6.49 in this melatonin treated group. Levels of aldosterone are also reduced by melatonin (-56.68 ± 7.62).

As Ogle and Kitay suggest (15), adrenal function is modulated by ovarian and pineal influences. Ovariectomy was therefore, performed in pinealectomized and un-pinealectomized rats in order to find if the renin-angiotensin system was altered by changes in adrenal mineralcorticoid secretion due to an ovarian mechanism.

Figure 2 shows the changes in PRA, aldosterone and corticosterone levels fol-

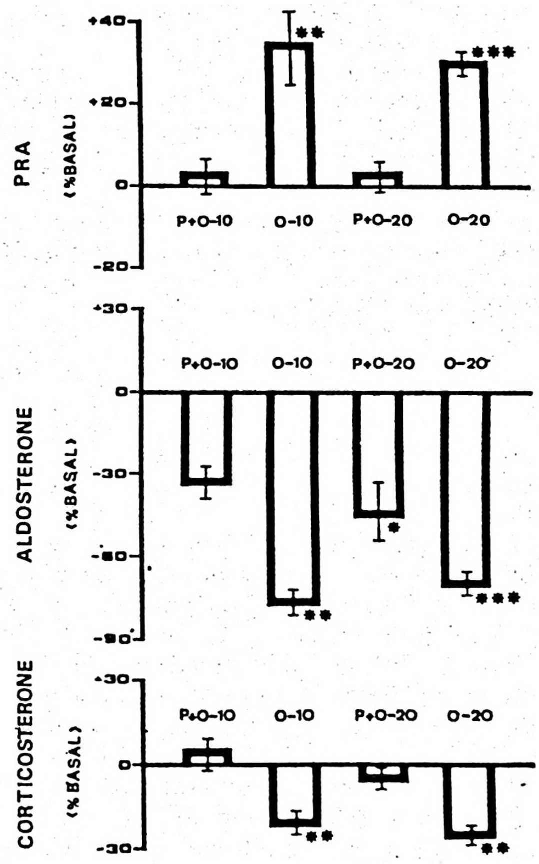

Fig. 2. *Effects of pinealectomy and/or ovariectomy on percentages of PRA, aldosterone and corticosterone basal levels in 10 and 20 day old groups.*
$p < 0.01$: ***; $p < 0.05$: **; $p < 0.1$: *.

lowing pinealectomy and/or ovariectomy (P + 0-10, P + 0-20, and 0-10 and 0-20 groups). Ovariectomy performed in pinealectomized rats prevents the increase in renin secretion, pinealectomy-dependent, at 10 and 20 days (P + 0-10: +2.19 ± 19.37; P + 0-20: +2.37 ± 7.88). In ovariectomized rats, however, PRA increases in two groups (0-10: +34.90 ± 12.79; 0-20: +30.87 ± 6.27).

Aldosterone significantly decreases in ovariectomized groups (0-10: —79.71 ± 5.27; 0-20: —71.14 ± 5.60). In groups subjected to both operations, aldosterone shows a slight decrease only 20 days after surgery (P + 0-10: —27.62 ± 10.92; P + 0-20: —42.97 ± 13.87).

Corticosterone levels and PRA change simultaneously in opposite directions. Ovariectomized rats undergo both a corticosterone decrease and a PRA increase (corticosterone: 0-10: —18.41 ± 4.20; 0-20: —22.27 ± 3.41). In other groups (i.e. pinealectomized and ovariectomized) no changes were found in either corticosterone or PRA levels.

Discussion

KARPPANEN *et al.* (8) show that pinealectomy induces an increase in plasma renin activity (PRA) through hyperactivity of the sympathetic nervous system, while basal levels of aldosterone remain similar to control sham-operated animals.

The results presented in figure 1 clearly show that PRA significantly decreases in all groups, while as KARPPANEN *et al.* suggest (8) the aldosterone levels do not change.

OGLE and KITAY (15) have demonstrated that the pinealectomy-induced corticosterone increase in female rats may be due to a decrease in adrenal 5-α-reductase activity.

The present results also show a post-pinealectomy corticosterone increase. Melatonin administration can reverse the pinealectomy-induced mineralcorticoid alterations. Melatonin decrease both corticosterone and aldosterone levels. The corticosterone decreased produced by melatonin treatment may be explained by an increase in its metabolization, since this pineal indol seems to enhance 5-α-reductase activity (15).

There is evidence to suggest that melatonin inhibits the progesterone metabolization to cortisol and aldosterone (13), which explain the aldosterone decrease following melatonin administration as recorded in figure 1.

Melatonin reverses the effects of pinealectomy on the renin-angiotensin system. Thus, PRA increased in this group, while corticosterone levels decreased. Melatonin, a pineal substance, appears to be at least partially responsible for the actions of the pineal gland on adrenal metabolism.

As OGLE and KITAY show (15) the pineal gland can modulate corticosterone secretion by both ovarian dependent and independent mechanisms. Consequently, cardiovascular homeostasis, dependent on the renin-angiotensin system, could be determined by changes in corticosterone levels that affect renin secretion.

Ovariectomy performed in pinealectomized rats prevents PRA decrease for at least 20 days postpinealectomy (fig. 2). Moreover, in this experimental group, ovariectomy prevents the corticosterone increase postpinealectomy, which remains at control levels. This effect on corticosterone secretion may be due to a lack of ovarian estrogen output. Estrogen (estradiol) is known to inhibit adrenal 5-α-reductase activity (15) and to increase hypophysarial ACTH secretion (1), which in turn enhances corticosterone secretion. Thus, the lesser in ACTH secretion and the lack of this estrogen inhibitory mechanism on reductase activity can explain the lower in corticosterone level following ovariectomy in rats.

Aldosterone drops slightly at 20 days

postovariectomy in pinealectomized rats (fig. 2), but as has been seen, PRA, as well as corticosterone remain at control levels. This suggests that corticosterone in rats is an important mineralcorticoid regulating the renin-angiotensin system.

Ovariectomy performed on control rats significantly decreases both corticosterone and aldosterone levels. After ovariectomy, however, PRA is significantly increased (fig. 2). These results can be explained because estradiol is known to inhibit adrenal reductase activity (15) and pineal gland activity (1), which increases reductase activity. Thus, the lack of estrogen following ovariectomy enhances adrenal reductase activity by these twofold mechanisms, disminishing corticosterone levels. If corticosterone inhibits renin secretion by its mineralcorticoid mechanism, the decrease in corticosterone secretion can enhance renin secretion explaining the PRA increase in ovariectomized rats.

The postovariectomy aldosterone decrease is more dificult to explain. Perhaps the decrease in corticosterone (by transformation in other metabolites), which is the main substrate of aldosterone production, could provoke the decrease in aldosterone levels.

In summary, the data suggest that the renin-angiotensin system in female rats can be regulated by pineal gland modulating adrenal mineralcorticoid secretion. The ovarian mechanism may be an important factor in this regulation, modulating pineal action on adrenal metabolism.

Resumen

Se estudia la influencia de la glándula pineal sobre el sistema renina-angiotensina en ratas hembras. La pinealectomía produce un descenso en la actividad plasmática de renina (APR) y un aumento en el nivel de corticosterona, sin variar el de aldosterona. La administración de melatonina revierte los efectos inducidos por la pinealectomía. La ovariectomía produce un aumento de la APR y un descenso en los niveles de aldosterona y corticosterona. Las ratas ovariectomizadas y pinealectomizadas no muestran cambios significativos en la APR ni en la corticosterona. Los resultados sugieren que la glándula pineal puede influir sobre el sistema renina-angiotensina por un mecanismo doble, ovárico y adrenal.

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