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Effects of Spreading Depression on Stress-Induced Changes in Plasma Prolactin and LH[1] (39004)

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The levels of prolactin and luteinizing hormone (LH) in blood are strongly influenced by anesthetics and other stressful agents or conditions (1–6). In previous reports (7–13) we have shown that, under continuous ether anesthesia, cortical spreading depression (SD), induced by applying concentrated KCl to the cerebral dura, results in elevating plasma prolactin in female rats pretreated with PMS and hCG, while in ovariectomized rats pretreated with estrogen and progesterone it raises plasma LH levels. In unanesthetized rats, pretreated according to these hormonal regimens and then subjected to the stress of a novel environment, further differences in responsiveness were noted (2). In view of these variances it seemed desirable to test the effect of cortical spreading depression on the prolactin and LH responses to recovery from ether anesthesia in a novel environment—following the surgery needed to implement the SD. The results indicate that SD inhibits the rise in plasma prolactin and the depression in plasma LH that would otherwise have followed the input of this complex of stressful stimuli.

Materials and methods. Adult (220–260 g) female Sprague–Dawley (Simonsen) rats were maintained on a standard laboratory diet and under controlled lighting with "lights on" 14 hr from 05:00–19:00. They were either ovariectomized upon arrival or kept intact and their vaginal smears taken daily. Both ovariectomized and intact animals received hormonal treatments identical to those reported previously (6, 9). In brief, ovariectomized (4–6 week) animals were injected with 50 μg of estradiol benzoate and 25 mg of progesterone and used 3 days later (OVX-E_2-P), whereas intact ones received a single injection of 300 IU of PMS (pregnant mare serum) and then 120 IU of hCG (human chorionic gonadotropin, Pregnyl, Organon) 54–58 hr later and were used 7 days after PMS (PMS-hCG).

On the day of experiment animals were rapidly anesthetized with ether and placed in a stereotaxic instrument; a small hole was drilled through each frontal bone. A cotton ball soaked in either physiological saline or 25% KCl was applied to the exposed surface of the dura, then covered with dental cement and the skin sutured. The whole procedure was completed in 7–10 min. The animals were then placed in separate containers in an isolated room and decapitated (within 15 sec of the operator's entering the room) 15, 30 or 60 min after the completion of surgical procedures. A third group of animals was decapitated immediately after surgery while still anesthetized (time zero). All the experimental series were completed between 11 AM and 1 PM.

Following decapitation trunk blood was collected and allowed to clot at +4° for 1–2 hr. After centrifugation the serum was separated and kept frozen prior to radioimmunoassay (RIA). LH and prolactin were measured in duplicate by RIA using the NIAMDD rat LH and NIAMDD rat prolactin systems, generously supplied by the Rat Pituitary Hormone Program of the NIAMDD, NIH. The reference preparations were NIAMDD-Rat LH RP-1 and NIAMDD-Rat Prolactin-RP-1 with a biological potency of 0.03 × NIH-LH-S1 (OADD assay) and 11 IU/mg (mouse deciduoma assay), respectively. Corticosterone was determined fluorometrically fol-

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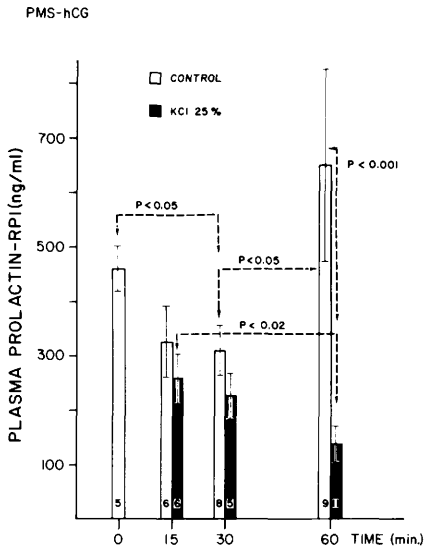

FIG. 1. Plasma prolactin levels in PMS-hCG control and 25% KCl-treated rats. Mean ± SE. Numbers within bars indicate number of animals. Included are *P* values of statistical significance in differences between groups.

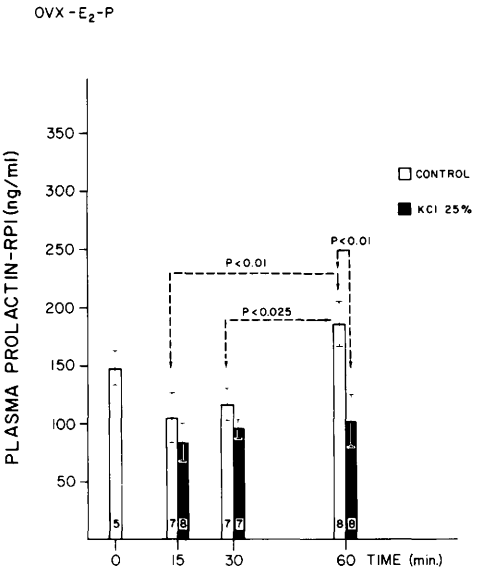

FIG. 2. Plasma prolactin levels in OVX-E_2-P control and 25% KCl-treated rats. Mean ± SE. For complete description see Fig. 1.

lowing essentially the method described by Glick *et al.* (14).

Group means were compared using Student's *t* test.

Results. Animals in which 25% KCl was applied were completely passive and lacking in motor activity when handled for decapitation.

Prolactin values in the PMS-hCG control group (Fig. 1) were lower at 15 and 30 min than at time zero or 60 min. The KCl group showed low levels at every other period when compared with time zero, with values progressively declining with time. The statistical significance of these differences is noted in Fig. 1. At 15 min, corticosterone levels were significantly higher (controls, 39.7 ± 3.6; KCl, 39.0 ± 2.9 μg/100 ml) than at time zero (9.2 ± 1.2 μg/100 ml), and at 60 min, still higher (controls, 50.8 ± 2.7; KCl, 54.4 ± 3.8 μg/100 ml) ($P < 0.001$ for time zero vs 15 or 60 min). In both groups, corticosterone levels were significantly higher at 60 than at 15 min ($P < 0.02$). In OVX-E_2-P animals prolactin levels (Fig. 2) in the control group also tended to drop at 15 and 30 min, when compared with time zero, and were again higher at 60 min. The KCl group showed fairly stable levels throughout the experimental period, at values not significantly different from those obtained at time zero.

Plasma LH values (Fig. 3) in control animals tended to drop at 15 min, rose significantly above 15-min levels at 30 min, but were markedly depressed at 60 min. This pattern of response is opposite from that observed for prolactin. In animals receiving KCl, LH values were stabilized at levels similar to the means observed at time 0, i.e., they were not depressed by the factors that induced the low LH levels in control animals.

Discussion. Our previous experiments were designed to study the effects of a fairly brief episode of spreading depression on plasma prolactin and LH levels that were remaining otherwise relatively constant (9, 10, 13). In the present experiments this was not the case, since "control" levels were changing significantly with time. Important differences between the current and earlier protocols include the briefer regime of ether anesthesia and the more prolonged application of KCl in the present study.

Earlier workers have reported both increases (1) and decreases (4) in plasma LH levels in response to ether anesthesia in ovariectomized rats, differences possibly at-

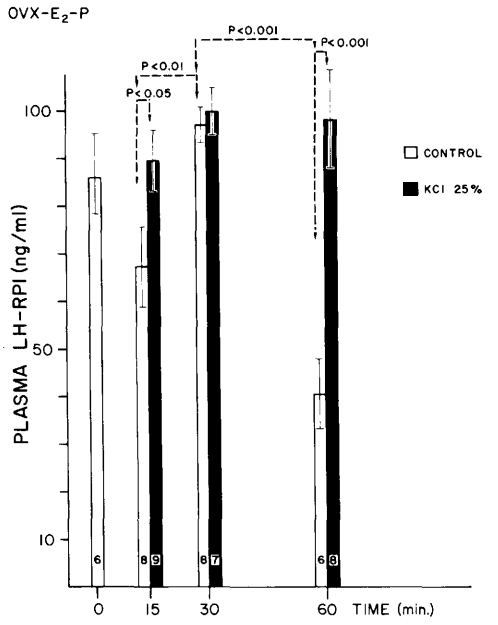

FIG. 3. Plasma LH levels in OVX-E_2-P control and 25% KCl-treated rats. Mean ± SE. For complete description see Fig. 1.

tributable to methods of blood sampling and application of the anesthetic. In our LH experiments we attempted to standardize the hormonal background conditions to a state optimal for LH release, with estrogen and progesterone (15, 12, 13), hormonal conditions under which stress of a novel environment lowered LH levels by 90 min (2). The LH values observed here at 7–10 min after exposure to ether and surgical procedures were comparable to and, if anything, lower than those reported previously. The apparently biphasic effect on LH observed here in "control" animals may represent responses to sequential stressing stimuli, i.e., initial ether and surgical stress followed by, as anesthesia wore off, pain and isolation in a novel environment.

With respect to prolactin, the patterns of response in OVX-E_2-P and PMS-hCG control groups were similar in direction if not in amplitude. Again the levels at time zero were comparable to those reported by Baldwin *et al.* (2) for the two hormonal backgrounds. In view of the fast responses of prolactin to stress (16) the "time-zero" values probably represent points in a decay curve from higher nonassayed levels. In the same way, the 15- and 30-min values may represent later points in the hypothetical decay curve, followed by a rise at 60 min related to secondarily acting stress factors.

When the animals were subjected to cortical SD just before time zero, quite different responses were observed to this composite stress situation, in both prolactin and LH. The marked fluctuations in LH and prolactin seen in OVX-E_2-P control animals were absent in SD-treated groups: The levels of both hormones remained stable at all three bleeding times. In other words, SD appeared to inhibit responses to secondarily acting stress stimuli such as the experience of pain and a novel environment on recovery from ether anesthesia. Furthermore, in the PMS-hCG animals under SD, blood levels of prolactin not only failed to increase but actually registered a continued decrease with time. This prolactin response pattern is opposite from our earlier finding in PMS-hCG animals under continuous ether anesthesia and sequential blood sampling (9) and more closely resembles unpublished observations in animals subjected to SD after bilateral amygdalectomy while under continuous ether anesthesia. Whether this represents a mere coincidence or a differential "tuning" of amygdalar input under the imposed experimental conditions remains a matter of speculation.

In an attempt to distinguish differences between control and KCl-treated groups with respect to stress responses, corticosterone levels were measured. In view of the dynamic of the corticosterone response to stress (17–19) and the lack of differences in the two groups, the results suggest that the corticosterone output was triggered by the initial ether and surgery and was not reflecting the ongoing stress situation.

The results suggest that cortical SD can suppress, at least in part, pituitary responses to stress. The general spread of depression through cortical areas including the pyriform and entorhinal cortices (20, 21), and the changes induced in thalamic activity (22), suggest that SD may alter perception of environmental variables and pain. Since SD also induces an indirect decrease in bioelectric activity of preoptic and hypothalamic areas (22, 11) as well as hormonal changes,

the results suggest that neuroendocrine effector as well as analyzer–integrator mechanisms are affected (see Ref. 23).

It seems apparent that factors arising from experimental procedures may exert profound effects on the direction and/or amplitude of hormonal responses to at least certain neurogenic inputs. This is illustrated by the effects of amygdalar stimulation on blood LH levels (24) and hippocampal stimulation on corticosterone (25). This plasticity of input (neural)–output (hormonal) relationships, at least when certain circuits are being considered, questions the attempts to label structures as either inhibitory or excitatory in nature.

Summary. Female rats rendered "pseudopregnant" by treatment with PMS and hCG and ovariectomized rats injected with estradiol and progesterone (OVX-E_2-P) were subjected to cortical spreading depression (SD). Within 7–10 min under ether anesthesia in a stereotaxic instrument a frontal craniotomy was performed and a cotton ball saturated with physiological saline (control) or 25% KCl was applied to the exposed dura, covered with dental cement and skin sutured. The animals were then placed in separate containers in an isolated room and decapitated for collection of trunk blood at 0, 15, 30, or 60 min after surgery. In PMS-hCG saline-treated control animals, prolactin levels had dropped by 15 and 30 min when compared with the zero-time values but by 60 min had increased significantly above the 30-min level. At that time (60 min), prolactin values in the KCl group were significantly lower than in the controls. Corticosterone levels were high at both 15 and 60 min in control and KCl groups. In OVX-E_2-P control animals, plasma prolactin levels also rose at 60 min compared with 15- and 30-min samples and at 60 min were significantly higher than in the KCl group. In control animals, LH levels were lower at 15 and 60 min than at zero time, but they remained unchanged in the KCl group. The data are interpreted as indicating that cortical SD suppresses the stress responses observed in saline-treated control animals.

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