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Effect of Methaqualone on Plasma Corticosterone in Rats: Possible Sites of Action

WILLIAM O. BOGGAN[1] AND JERROLD S. MEYER[2]

Department of Psychiatry and Behavioral Sciences
Medical University of South Carolina, Charleston, SC 29425

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BOGGAN, W. O. AND J. S. MEYER. *Effect of methaqualone on plasma corticosterone in rats: Possible sites of action.* PHARMAC. BIOCHEM. BEHAV. 16(6) 925–927, 1982.—Methaqualone produces large increases in plasma corticosterone in rats. This effect does not involve a direct stimulation of either the adrenal glands or pituitary, nor is it the result of a significant alteration in the clearance of circulating corticosterone. Methaqualone may therefore be influencing brain mechanisms which ultimately regulate hypothalamic CRH secretion, although a peripheral site of action has not yet been ruled out.

Methaqualone Corticosterone Pituitary-adrenal system Sedative-hypnotic

METHAQUALONE (2-methyl-3-ortho-tolyl-4(3H)-quinazolinone) is a non-barbiturate sedative-hypnotic which possesses potent anticonvulsant properties [2,7] and which also disrupts temperature regulation in experimental animals [1,11]. Although the influence of methaqualone on hormonal function is still largely unknown, we have previously reported a drug-induced stimulation of pituitary-adrenocortical activity in mice [8]. This effect of methaqualone was essentially independent of its hypothermic action, but could be completely blocked by pretreatment with the potent glucocorticoid dexamethasone. Methaqualone therefore did not seem to be acting directly on the adrenal cortex, but it was still unclear as to whether the drug might be influencing the pituitary-adrenal system at some other level.

The present series of studies was therefore designed to test the possibility that methaqualone-induced elevations of plasma corticosterone might be the result of either a direct stimulation of pituitary adrenocorticotropic hormone (ACTH) secretion or a reduction in the rate of corticosterone clearance from the circulation. Because these studies used rats as subjects, additional experiments were also performed to verify some of our earlier findings obtained in mice.

METHOD

The animals were male Sprague-Dawley rats (300–350 g) which were individually housed and allowed access to standard rat chow and tap water ad lib. The colony room was maintained under 12:12 light-dark cycle (lights on at 0700 hr) and a temperature of approximately 22°C.

Experiment 1: Pituitary-Adrenal Response to Methaqualone

Previous studies concerning the effect of methaqualone on circulating corticosterone concentrations had been conducted utilizing mice as subjects [8]. Because the experiments described below required the use of rats instead, the first priority was to demonstrate that methaqualone also increases plasma corticosterone in the latter species. The drug was made up as a suspension in 1% gum acacia in view of its virtual insolubility in water. Rats were injected intraperitoneally with either 75 mg/kg methaqualone or the gum acacia vehicle. This dose of the drug produced moderate sedation in the animals as indicated by a somewhat ataxic walk. Blood was then collected into heparinized syringes 1 hr later via cardiac puncture under ether anesthesia. Dose and time parameters were chosen on the basis of our earlier work to yield a large pituitary-adrenal response. Plasma samples were assayed for corticosterone by a previously described competitive protein-binding radioassay [8,9].

Experiment 2: Effect of Hypophysectomy on the Pituitary-Adrenal Response to Methaqualone

Rats were parapharyngeally hypophysectomized by a vendor (Hormone Assay Laboratories), shipped to us, and then tested on the 4th day following surgery. While in our laboratory they were maintained on rat chow and a 5% sucrose solution. Three groups of operated rats were used, two of which received either 75 mg/kg methaqualone or gum

[1]Send reprint requests to Dr. W. O. Boggan, 508 Research Building, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.

[2]Present address: Department of Psychology, University of Massachusetts, Amherst, MA 01003.

acacia as before. The third group was injected subcutaneously with 4 IU of ACTH (ACTHAR Gel, Armour), thus serving as a control to demonstrate that the adrenal cortex was still responsive at this time interval following hypophysectomy. Cardiac puncture was performed 1 hr after injection in each case, and the resulting plasma samples assayed for corticosterone.

Experiment 3: Effect of Basal Hypothalamic Lesions on the Pituitary-Adrenal Response to Methaqualone

Rats were given large lesions of the basomedial hypothalamus in order to destroy the neurohemal contact zone (i.e., median eminence) where endogenous corticotropin releasing hormone (CRH) is secreted into the pituitary portal system. These animals were then used to determine whether methaqualone could cause a direct release of ACTH from the pituitary, as indicated by changes in circulating corticosterone levels. Bilateral lesions were performed under pentobarbital anesthesia by passing 6 mA of cathodal current for 17 sec through stainless steel electrodes which were completely insulated except for a length of 0.5 mm at the tip. The stereotaxic coordinates were 5.6 mm anterior to the ear bars, ±0.6 mm lateral from the midline, and 0.2 mm above the base of the skull (the electrode was first lowered down to the base of the skull and then elevated the required distance).

Basomedial hypothalamic lesions may initially disrupt the pituitary portal circulation, thus diminishing the ability of blood-borne substances to reach the secretory cells of the adenohypophysis. However, Porter *et al.* [10] found evidence for a revascularization within 2 days following lesions similar to ours. The testing sequence for each rat was therefore begun on the second day after surgery. At this time, the animals were subjected to an initial cardiac puncture under ether anesthesia, followed by a second blood drawing 15 min later. The first sample yielded baseline corticosterone concentrations, while the second served as an index of the pituitary-adrenal response to ether stress. This response is almost completely blocked by lesions which have effectively destroyed the median eminence [13], hence we were able to prescreen our animals by discarding any which displayed poststress values in excess of 10 μg/100 ml plasma.

The actual methaqualone test was performed on day 3 following the lesion. Rats were injected with 75 mg/kg of the drug, blood obtained 1 hr later by cardiac puncture, and the samples analyzed for corticosterone. A final control procedure was then carried out on day 4. Each animal was injected intravenously (IV) with a crude CRH preparation obtained by homogenizing rat hypothalami in ice-cold 0.1 N HCl, centrifuging, and then neutralizing the supernatant with 0.1 N $NaHCO_3$ [3]. A dose equivalent to 0.5 hypothalamus/subject was utilized, and blood was drawn 15 min after the injection for subsequent corticosterone assay. This CRH challenge was used to demonstrate that the pituitary-adrenal system was still functional and responsive in our experimental animals. After all testing was completed, the rats were anesthetized, perfused transcardially with Formalin, and the location of each lesion verified using standard histological methods.

Experiment 4: Corticosterone Clearance in Methaqualone-Treated Rats

The purpose of this experiment was to determine whether the elevation in plasma corticosterone observed after methaqualone could be due to a decreased clearance of corticosterone from the circulation. Two groups of rats were utilized, one of which was given 75 mg/kg methaqualone while the other received gum acacia only. Fifteen minutes after these treatments, all animals were injected IV with 0.5 μCi of (1,2-^{3}H)corticosterone (40 Ci/mmole, New England Nuclear). Blood samples were then obtained from each animal at 15, 30, and 45 min after the label injection. Plasma was prepared from each sample and then extracted with 6 volumes of dichloromethane. The resulting extracts were dried under nitrogen, redissolved in absolute ethanol, and finally counted. In order to verify the nature of the extracted radioactivity, separate aliquots of each sample were mixed with unlabeled corticosterone and then chromatographed on silica gel F-254 sheets (Eastman Kodak) using a solvent system of benzene-ethanol 9:1. Spots were visualized under UV light, eluted with absolute ethanol, and counted as before.

TABLE 1

PLASMA CORTICOSTERONE RESPONSE TO METHAQUALONE IN INTACT, HYPOPHYSECTOMIZED, AND HYPOTHALAMIC-LESIONED RATS

Group	Plasma Corticosterone Concentration (μg/100 ml)
Experiment 1 (Intact)	
Gum acacia (6)	3.8 ± 1.2*
Methaqualone (6)	40.9 ± 7.6
Experiment 2 (Hypox)	
Gum acacia (6)	N.D.†
Methaqualone (6)	N.D.
ACTH (6)	38.7 ± 1.8
Experiment 3 (Lesioned)	
Baseline (5)	1.3 ± 0.4
Ether	4.2 ± 0.6
Methaqualone	4.1 ± 0.9
CRH	13.1 ± 2.0

*Values displayed = mean ± SEM.
†N.D. = "not detectable."
The number of animals in each group is shown in parentheses. Note that the same 5 animals were used for all treatments in Experiment 3.

RESULTS

The results of Experiments 1–3 are shown in Table 1. Methaqualone at a dose of 75 mg/kg was found to increase significantly plasma corticosterone levels measured 1 hr after drug administration (Experiment 1, $p<0.001$ by t-test). Short-term hypophysectomy (Hypox) totally abolished the response to methaqualone (Experiment 2). Yet the adrenal cortex was still functional in these animals as indicated by the marked effect of exogenous ACTH on circulating corticosterone concentrations. This provides strong evidence that methaqualone does not stimulate pituitary-adrenal activity by acting on the adrenal gland itself.

Experiment 3 was designed to investigate whether methaqualone might be directly influencing pituitary ACTH secretion. Rats were given basomedial hypothalamic lesions aimed at destroying the median eminence region and thereby preventing the release of endogenous CRH. Based on their post-lesion reactivity to ether stress, five animals were selected for use in the experiment. As shown in Table 1,

these animals displayed only a slight elevation in plasma corticosterone after ether administration. Although this residual response was statistically significant (Ether vs Baseline, $p<0.01$ by paired t-test), the fact that intact rats treated in the same manner typically display corticosterone concentrations in excess of 30 μg/100 ml [4] demonstrates the successful placement of our lesions. Postmortem histological examination of each brain further verified that the median eminence and a large surrounding area were totally eliminated in all cases.

The corticosterone response to methaqualone was similar to that following ether, both in terms of its absolute magnitude and because it also attained statistical significance (Methaqualone vs Baseline, $p<0.05$). However, it remains clear that the hypothalamic lesions severely blunted the normal response to this drug (compare methaqualone groups in Experiments 1 and 3) without compromising the ability of natural CRH to stimulate pituitary-adrenal activity (CRH vs any other condition, all $ps<0.01$). Thus, it can be concluded that methaqualone does not act like a CRH by directly promoting pituitary ACTH secretion.

The final experiment examined the effect of methaqualone on the clearance of (^{3}H)corticosterone from the circulation. Serial blood samples obtained from gum acacia or methaqualone-treated rats were extracted (see Method section), counted, and the raw counts subjected to a logarithmic transformation. Thin-layer chromatography showed that 70–90% of the extracted radioactivity co-migrated with authentic corticosterone, suggesting that most of the circulating label was still in its original form. The transformed data were plotted against time and the best-fit line for each animal calculated by means of a linear regression analysis. The slope of each line was then used to compute a corticosterone half-life ($t_{1/2}$) for that animal. Based on this procedure, the methaqualone group displayed a somewhat longer ($t_{1/2}$) than did the controls (18.8±2.5 min vs 15.2±1.6 min, mean±SEM for 7 animals/group); however, this effect was nonsignificant ($p>0.05$ by t-test) and was too small in any case to be solely responsible for the large, rapid corticosterone rise which occurs following methaqualone administration. Hence, this rise must principally be due to a stimulation of corticosterone secretion rather than an inhibition of its degradation and elimination.

DISCUSSION

The present results have shown that methaqualone produces large increases in plasma corticosterone concentrations in rats, just as it does in mice [8]. Methaqualone does not appear to act at the level of either the adrenal or pituitary gland, nor does it have a major effect on the clearance rate of circulating corticosterone. It may be the case that one or more of the drug's peripheral actions result in a "stress response," thus stimulating pituitary-adrenal activity only secondarily. Nonetheless, the widespread uptake of methaqualone into brain [12] as well as its well-known effects on central processes [1, 2, 7, 11] leave open a strong possibility that the drug may interact with neural pathways directly involved in endocrine regulation.

Many psychoactive drugs covering a wide spectrum of activity are known to provoke substantial pituitary-adrenal responses (e.g., see [14]). It is particularly interesting, however, that behaviorally depressant drugs such as methaqualone ([8] and this paper), ketamine [6], and ethanol [5] can have such powerful effects in this regard. Further studies will be necessary to elucidate the exact mechanisms by which these substances act to influence various behavioral and physiological functions.

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