ANTAGONISTIC EFFECT OF METHADONE ON STEROIDOGENESIS AND THE ADENYLATE CYCLASE SYSTEM IN ISOLATED RAT ADRENOCORTICAL CELLS

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SUMMARY: Methadone exhibits an antagonistic effect toward steroidogenesis which lies prior to progesterone in the biosynthetic pathway in isolated rat adrenal cells. Levels of adenosine cyclic 3'-5' monophosphate are depressed in a dose dependent fashion in ACTH stimulated cells as is steroidogenesis in cells stimulated with N^6-O^2-dibutyryl adenosine cyclic 3'-5' monophosphate. Stimulation produced by the ACTH analog, O-nitrophenyl sulfenyl ACTH, is also inhibited by methadone. The participation of adenosine cyclic 3'-5' monophosphate as an obligatory messenger in ACTH stimulated steroidogenesis is discussed with respect to the pharmacological properties of methadone in this system.

The physiological sequelae of methadone use are poorly understood. It, like other narcotic analgesics, has been pictured as interferring with the release of corticotrophin releasing factor and thereby depressing the response of the Hypothalmic-Pituitary-Adrenal axis (1). Investigations exploring any direct effects of methadone on adrenal steroid biosynthesis, however, have been neglected. We recently showed that methadone antagonized both corticosterone and aldosterone production in a dose dependent manner (2).

One potentially important site for methadone antagonism is the putative second messenger system, cAMP (3). Since the most important site of antagonism appears to be prior to

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Abbreviations: cAMP for adenosine 3'-5' cyclic monophosphate, dbcAMP for N^6-O^2-dibutyryl adenosine 3'-5' cyclic monophosphate and NPS-ACTH for O-nitrophenyl sulfenyl ACTH.

progesterone, we decided to investigate the effect of methadone upon cAMP.

METHODS: Adrenal cortical cells were isolated from 150-200 gm male Sprague-Dawley rats by the technique of Sayers' group (3,4). Then 0.9 ml of adrenal cell suspension containing 60,000 cells plus or minus 10 percent was pipetted into silanized 25 ml erlynmeyer flasks, equipped with rubber septa. At this point either 0.05 ml aliquots containing methadone, ACTH, or buffer alone were added to a final incubation volume of 1.0 ml. The vessels were incubated under a 95% O_2 5% CO_2 atmosphere for 1.0 hour after which the contents of each vessel was snap frozen in a sample vial cooled in dry ice-acetone.

Corticosterone concentration was measured by radioimmunoassay (2).

Progesterone concentration was measured by radioimmunoassay using a hexane extraction of 0.05 ml of adrenal cell incubate added to 0.5 ml of distilled water. Recovery from the extraction was estimated by the addition of (1-2^{3}H) progesterone. The hexane extract was dried and reconstituted to 0.5 ml in ethyl acetate and duplicate 0.05 ml sample dryed and assayed by specific radioimmunoassay using antiserum to 11-α hydroxy progesterone hemisuccinate, while 0.10 ml was counted for recovery purposes (5).

cAMP concentrations were determined by the preparation of an ethanol filtrate followed by specific radioimmunoassay. To 1.0 ml of absolute ETOH, 0.250 ml of adrenal incubation mixture was added. The mixture was vortexed vigorously and centrifuged. Duplicate 0.5 ml of aliquots of ETOH were added to assay tubes and dried in a vacuum. The quantity

of cAMP was then measured by radioimmunoassay using a specific antiserum (less than 0.00001% crossreactivity with other adenine nucleotides) and (^{3}H) cAMP obtained from New England Nuclear (6).

RESULTS AND DISCUSSION: As summerized in Figure 1, methadone

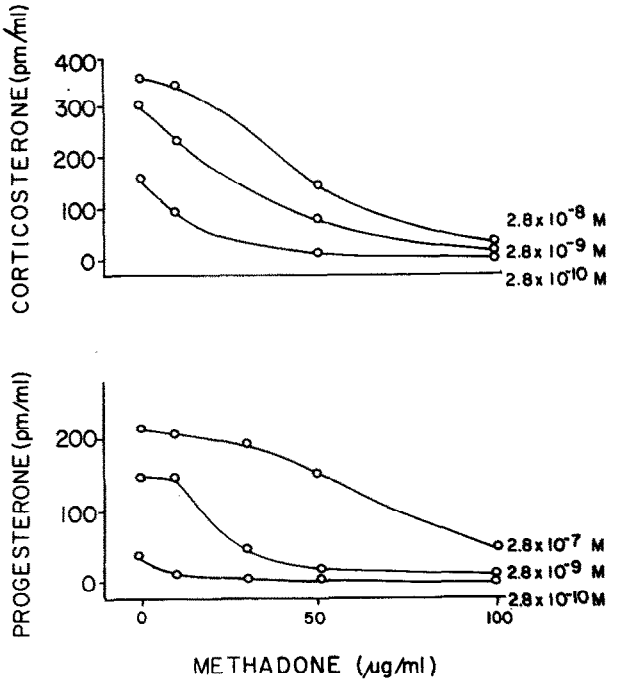

Fig 1. Dose dependent inhibition of ACTH stimulated Corticosterone and Progesterone production by methadone with varying concentrations of ACTH.

inhibits adrenal steroidogenesis in a dose dependent fashion. The character of the inhibition clearly indicates that the mechanism involved contains a non-competitive component that is asserted to a greater degree the larger the concentration of methadone employed in the incubation medium, and that the mixed inhibition characteristic of methadone antagonism may indicate the involvement of more than one biochemical event.

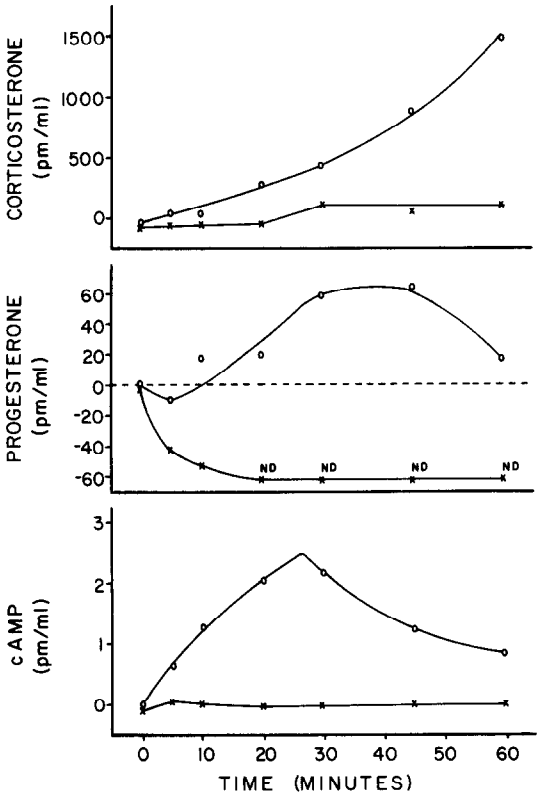

Fig 2. Accumulation of Corticosterone, Progesterone, and cAMP in adrenal cells stimulated by 2.8 x 10^{-9}M ACTH in the presence (x) and absence (o) of 100 µg/ml methadone.

Figure 2 shows a time study in which adrenal cells were batch stirred with a polypropylene paddle. Corticosterone production showed a significant response within 5 min after the addition of 1000 µU/ml ACTH. Corticosterone continued to accumulate at a slightly accelerating rate through-out the incubation. Progesterone increased significantly beginning at 10 min and reached a plateau at 30 min and declined by the end of the incubation. Production of cAMP increased rapidly with the first significant increase observable by 2 min. It reached a plateau by 20 min and showed a steady decline beginning at 30 min but failed to reach basal

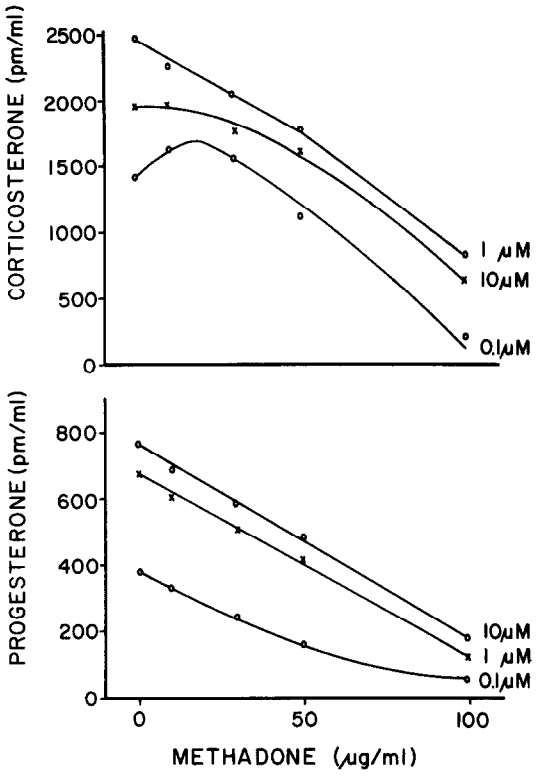

Fig 3. Dose dependent inhibition of dbcAMP stimulated Corticosterone and Progesterone production by methadone with 0.1 μM, 1 μM, and 10 μM concentrations of dbcAMP.

levels at the end of incubation. In an incubation in which methadone was incorporated (100 μg/ml), there was no significant increase in corticosterone production through the first 20 min. By 30 min corticosterone increased by 185 pm and remained relatively constant through the remainder of the incubation. Progesterone which had an initial concentration of 0.02 pm/ml, decreased by the 20th min to undetectable levels, suggesting that methadone's disruption of adrenal steroid biosynthesis is prior to progesterone in the biosynthetic pathway. In contrast to the control incubation, no cAMP production was detectable (<0.3 pm/ml) in the presence of 100 μg/ml methadone.

Figure 3 shows the effect of methadone on dbcAMP stimulated steroidogenesis. It can be seen that methadone is potent antagonist in this system as well. If one assumes that dbcAMP is equivalent in its action to endogenous cAMP, then it is reasonable to conclude that methadone exerts an inhibitory effect at a point subsequent to cAMP formation.

In our hands no increase in cAMP was detected with

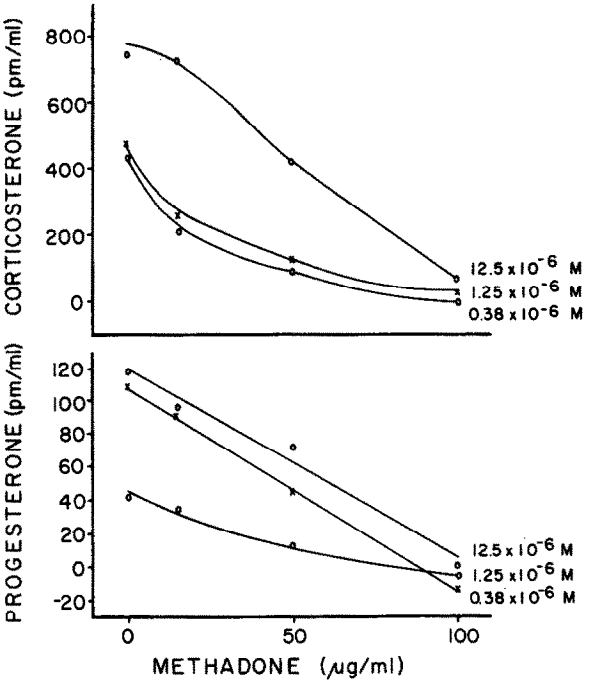

Fig 4. Dose dependent inhibition of NPS-ACTH stimulated Corticosterone and Progesterone production by methadone with varying concentrations of NPS-ACTH.

NPS-ACTH (7). Figure 4 shows that NPS-ACTH stimulated corticosterone production was inhibited by methadone. The ED_{50} for corticosterone was 19 µg/ml of methadone, which cannot be differentiated from the ED_{50} of 21.4 µg/ml of methadone with native ACTH that we reported previously (2).

Furthermore, the sensitivity of cAMP accumulation to methadone is directly proportional to the dose of ACTH (Table I). If the increased sensitivity of the system to

TABLE I

Relationship between ACTH concentration and dose of methadone required to provide 50% inhibition (ED_{50}) of cAMP

[ACTH]	ED_{50} (Methadone)
2.8×10^{-9}M	22 µg/ml
2.8×10^{-7}M	64 µg/ml

methadone antagonism with lower doses of ACTH is due to a decrease in cAMP formation then one would expect a greater sensitivity of steroidogenesis to methadone antagonism with NPS-ACTH since cAMP production is considerably lower. Since there was no change in the sensitivity of the system to methadone, these data are not consistent with the postulate of cAMP as the obligatory second messenger to ACTH stimulation. It must be emphasized that our interpretation is tentative due to our lack of sufficient knowledge of the specifics of methadone effects upon both the metabolism of cAMP and steroidogenesis per se.

These studies have relevance from the standpoint of providing an understanding of the pharmacology of methadone which maybe applicable to other tissues, most notably the testes, since it has been shown that methadone decreases testosterone in spite of a concommitant increase in LH (8).

They also show that methadone may prove to be a valuable probe in resolving questions of cAMP as the second messenger for ACTH. It would also be logical to extend the studies of effects of methadone on the adenyl cyclase system to a primary organ of medical interest, i.e. the brain.

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