STUDIES ON THE PHYSICAL DEPENDENCE LIABILITY OF ANALGESICS

4TH REPORT: ELECTRON MICROSCOPIC STUDY ON INTRAMITOCHONDRIAL STRUCTURE OF ZONA FASCICULATA CELLS IN ADRENAL CORTEX IN CODEINE ADDICTED RATS

Ichiro YANO, Yasusuke MASUDA, Hitoo NISHINO, Hiroyuki YAMAMOTO and Tadashi MURANO

Department of Pharmacology, Wakayama Medical College, Wakayama, Japan

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Abstract - Influence of codeine on the fine structure of mitochondria in the cells of zona fasciculata of rat adrenal cortex was studied electronmicroscopically. By the administration of codeine in a dose of 40 mg/kg, the number of intramitochondrial vesicles was decreased, the change being diminished during repeated administration of the drug. Withdrawal of codeine reproduced an extensive transformation, while with a re-injection of the drug, a prompt restoration to the pretreated structure was observed. The feasibility of utilizing this transformation as an index of physical dependence liability has been discussed.

It has been evidenced that abrupt withdrawal of morphine or levallorphan challenging produces a marked transformation of ultrastructure of zona fasciculata mitochondria in adrenal cortex of the morphine addicted rats (1) and the time course of appearance of the transformation is in close agreement with that of loss in body wt. after withdrawal, this being the best index of physical dependence liability. On the other hand, CG-315, a newly synthesized analgesic, shows neither the mitochondrial transformation nor fall in body wt. after withdrawal of the drug during chronic administration (2, 3).

The present experiments were undertaken to clarify whether codeine with moderate physical dependence liability (4, 5) is able to produce the mitochondrial transformation after withdrawal.

MATERIALS AND METHODS

Unless otherwise noted, the experimental rat strain, feeding condition and procedures for electron-microscopic examination were the same as described in the first report (1).

In chronically administered experiments, rats were given four s.c. injections of codeine daily 6 hr intervals (6 a.m., noon, 6 p.m. and midnight) for 50 days. The initial dose of the drug was 20 mg/kg/day during the first week (5 mg/kg x 4 daily), 40 mg/kg in the second week, 60 mg/kg in the third week, 80 mg/kg in the 4th week and 100 mg/kg in the 5th week until sacrifice.
Codeine phosphate was dissolved in 0.1% acetate buffer (pH 7.1) and control group was given an injection of 0.2 ml/100 g of the buffer solution under the same schedule.

Fig. 1. Electron microscopic findings of zona fasciculata in adrenal cortex of normal rats. Mt: mitochondria, ER: endoplasmic reticulum, Ly: lysosome, Nu: nucleus. × 20000

Fig. 2. 12 hr after a single injection of codeine (40 mg kg, s.c.). Decrease in number of intramitochondrial vesicles was obvious. × 25000
RESULTS

1) Influence of a single administration of codeine

In normal rats, numerous nearly spherical mitochondria approx. 1.2–1.4 μ in diameter were seen in the cells of zona fasciculata. These mitochondria were demarcated by a double membrane. Small vesicles with an average diameter of about 500 Å were tightly packed in the matrix of the mitochondria, in a honeycomb like structure (Fig. 1).

Although no significant changes were observed 3 hr after codeine (40 mg/kg, s.c.) administration, the number of intramitochondrial vesicles diminished and the density of matrix decreased in 6–12 hr (Fig. 2), these changes being somewhat different from those in morphine treated rats which was transformed to tubular forms with increase in matrix density. The change of mitochondria above noted returned to a normal state 24 hr after administration. However, no marked change was observed in endoplasmic reticulum, nuclei and lysosomes.

2) Influence of chronic administration of codeine

When administration of codeine was repeated, the structural change of mitochondria seen after a single injection of the drug gradually diminished and the mitochondria was filled with microvesicles (Fig. 3).

After abrupt withdrawal, however, the number of vesicles began to diminish in 12 hr,
Fig. 4. Fine structure of adrenal cortex in codeine addicted rats 12 hr after withdrawal. ×25000

Fig. 5. Fine structure of adrenal cortex in codeine addicted rats 24 hr after withdrawal. ×25000
FIG. 6. Percent of abnormal mitochondria in the adrenal cortex of codeine addicted rats after withdrawal.

FIG. 7. Changes of mitochondrial fine structure 6 hr after administration of levalloorphin to codeine addicted rats. ×25000
as shown in Fig. 4, unlike the case of morphinization. In addition, after 24 hr, when the fall in body wt. reached a maximum state, the mitochondria also exhibited striking changes, i.e. remarkable diminution of the number of vesicles, followed by complete disappearance of honeycomb-like structure (Fig. 5).

Twenty-four hr after withdrawal when the change in mitochondria had reached a peak, re-administration of codeine resulted in the return to pretreated structure within a few hr.

Unless the withdrawal was interrupted by re-administration of codeine, the changes in mitochondria were still evident 36 hr after withdrawal, with amelioration in 48 hr and restoration in 72 hr (Fig. 6).

Administration of levallorphan to codeine chronically treated rats also produced the mitochondrial change similar to that above noted, although the time required for appearance of the change was shorter than that after withdrawal of codeine (Fig. 7).

DISCUSSION

When codeine was administered to rats, intramitochondrial vesicles of zona fasciculata of adrenal cortex diminished in number with decrease in matrix density 6-12 hr after administration, these morphological changes being somewhat different from those in morphine injected rats which were transformed into tubular forms with increase in matrix density (1).

In so far as codeine administration was uninterrupted in the drug chronically treated rats, the usual structure of mitochondria was kept. Abrupt withdrawal of the drug or administration of levallorphan reproduced the change in mitochondrial structure which appeared in case of a single injection of codeine, and corresponded to the fall in body wt., this resembled that in Actinomycin D (1, 6) or Amphenone B (7) treated rats and re-administration of codeine to withdrawn rats resulted in a return to the pretreated structure.

A satisfactory explanation for the differences between morphine and codeine in morphological changes of intramitochondrial structure, however, cannot be given only from the results of this experiment.

It was evidenced however, that 1) in codeine chronically administered rats, the time course of appearance of diminution of intramitochondrial vesicles in number after withdrawal is in close agreement with that of loss in body wt. which is the best index of physical dependence in rats similar to that seen in morphine addicted rats. 2) Levallorphan challenging produces a change similar to that after withdrawal. 3) After withdrawal, re-administration of codeine results in a return to the pretreated structure within a few hr similar to that seen in morphinized rats. 4) CG-315 shows neither transformation nor loss of body wt. after withdrawal in chronically administered rats.

From these results, the morphological change in mitochondria after abrupt withdrawal of analgetics in chronically administered rats may be used as an index of physical dependence liability in rats.

Finally, the results obtained in a series of these experiments (1, 3) are summarized schematically in Fig. 8.
MITOCHONDRIAL CHANGES WITH CODEINE

Fig. 8. Mitochondrial changes by various treatments.

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