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EFFECTS OF ACUTE AND CONTINUOUS MORPHINE ADMINISTRATION ON SERUM GLUTAMATE OXALACETATE TRANSAMINASE AND GLUTAMATE PYRUVATE TRANSAMINASE ACTIVITIES IN THE MOUSE

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Abstract—Mice were exposed continuously to morphine by implanting morphine pellets for 12 hr to 3 days. In mice receiving prolonged morphine pellet implantation, second and third morphine pellets were implanted at days 3 and 6, for a total of 9 days. Results showed morphine pellet implantation for 24 hr caused a 2-fold increase in glutamate oxalacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) activities, as compared with those of the placebo control group. SGOT and SGPT activities were returned to control levels after 3–9 days of morphine pellet implantation. In mice implanted with a morphine pellet for 24 hr, both SGOT and SGPT activities returned to normal levels at 3 and 6 days after the removal of the morphine pellet. In mice receiving morphine pellet implantation for 24 hr with three concurrent administrations of naloxone, 40 mg/kg, s.c., the morphine pellet-induced elevations of SGOT and SGPT activities were attenuated significantly. Acute administration of morphine sulfate by both subcutaneous and intraventricular administration elevated SGOT and SGPT activities. The elevations of SGOT and SGPT activities caused by morphine pellet implantation were prevented completely by hypophysectomy and prevented partially by adrenalectomy. These results substantiate our previous morphological and biochemical findings that initial administration with morphine by either injection or pellet implantation may alter hepatic function and the action may be mediated through the central nervous system.

Elevation of serum glutamate oxalacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) levels has been regarded as a diagnostic index of hepatic disease or myocardial infarction [1]. In terms of narcotic abuse, the pathogenesis of the liver dysfunction is much debated. Many narcotic addicts have a long-term intermittent serum hepatitis. Earlier studies (1920–1950) assumed a direct hepatotoxic effect of opiate and it was even suggested that elevated transaminase levels were a valuable adjunct in the diagnosis of heroin and cocaine use [2]. The morphologic changes may also represent a direct toxic effect of narcotics on the liver [3]. In a detailed study by Gorodetzky *et al.* [4], however, it was concluded that serum hepatitis of chronic narcotic addicts was caused by contamination of the hypodermic needles. Recent studies on comparison of liver function in intravenous users, smokers and sniffers of heroin also provided additional evidence that the liver disease of narcotic addicts was due to the injection of foreign materials rather than to opiates *per se* [5]. However, the high incidence of transaminase abnormalities in patients who denied ever using intravenous drugs makes it impossible to exclude fully a direct toxic effect of opiates [5].

Recent studies in our laboratory have demonstrated that morphine can inhibit microsomal drug-metabolizing enzyme activity in both male and female mice [6, 7]. The morphological findings from our laboratory also demonstrated that the liver was filled with round lipid droplets after subchronic exposure to morphine [8, 9]. These ultrastructural changes have also been confirmed with biochemical analysis [10]. It has also been shown that morphine pellet implantation decreased significantly the weight of the liver and the ratio of liver weight to body weight [11]. Further studies also demonstrated that CCl_4 potentiated toxicity in the mouse in which CCl_4 was concomitantly administered with subacute morphine administration [12].

In view of the pros and cons of narcotic drug effects on liver, it is the objective of this study to re-examine systematically the effect of morphine on SGOT and SGPT in the mouse by both morphine pellet implantation and acute injection of morphine sulfate, subcutaneously and intraventricularly.

MATERIALS AND METHODS

Male ICR mice weighing 25 ± 3 g (Charles River, Wilmington, MA) were used in various experiments. Hypophysectomized and adrenalectomized male ICR mice of the same size were also obtained from the same vendor. Animals were maintained on standard laboratory chow and tap water and were housed in a room lighted artificially for 12 hr of the day. Physiologic saline was used as drinking water in hypophysectomized and adrenalectomized animals. In each experiment, blood collected from eight mice per group was heparinized and serum was obtained by centrifugation.

Measurement of SGOT and SGPT activities. SGOT and SGPT activities were determined using reagent kits (No. 55-10 and 55-10 P) purchased from the Sigma Chemical Co., St. Louis, MO. Briefly, the procedures were essentially the original spectrophotometric methods of Karmen [13] and Wroblewski and LaDue [14]. In these methods the transaminase reactions were coupled to specific dehydrogenases so that the keto-

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acids resulting from the transamination were reduced to their corrresponding hydroxy acids by means of NADH. NADH was then oxidized to NAD and the resulting decrease in absorbance at 340 nm was measured with a Cary 219 spectrophotometer. Protein concentration was determined by the procedure of Lowry *et al.* [15].

Enzyme activity was calculated both in terms of Karmen units/mg of protein and Karmen units/ml of serum. One Karmen unit of SGOT or SGPT activity was defined as that amount of enzyme which caused a decrease in A_{340} of 0.01/min in a 3-ml volume at 25°. This was equivalent to the formation of 4.82×10^{-4} μmoles oxalacetic acid or pyruvic acid/min.

In entire experiments, SGOT and SGPT activities in vehicle or placebo control animals were between 183 ± 11.4 units/ml or 3.08 ± 0.22 units/mg of protein and 40 ± 2.5 units/ml or 0.67 ± 0.04 units/mg of protein respectively. Both SGOT and SGPT activities of morphine-treated animals presented in the figures were expressed as per cent of corresponding control values. Statistical analysis for significance was checked by Student's *t*-test and the P values are shown in figures.

Effects of continuous administration of morphine on SGOT and SGPT activities. In the first experiment, mice were exposed continuously to morphine by implanting a morphine pellet [16] for 1 or 3 days. In mice receiving prolonged morphine pellet implantation, second and third pellets were implanted at days 3 and 6 for a total of 9 days. In the second experiment, mice were implanted with a morphine pellet for 24 hr. One-half of the morphine pellet-implanted animals were deprived of food and water for the same period of time. The control animals were devided similarly except that a placebo pellet was implanted. In the third experiment mice were implanted with a morphine pellet for 24 hr. SGOT and SGPT activities were determined at different times (between 0 and 6 days) after pellet removal. The schedule was planned so that all groups were killed on the same day. In the fourth experiment, two groups of mice were implanted with a morphine pellet for 12 and 24 hr respectively. Another group of mice was implanted with a morphine pellet for 24 hr with three concurrent administrations of naloxone, 40 mg/kg, s.c., at 8-hr intervals.

In the fifth experiment, adrenalectomized or hypophysectomized mice were implanted with either a morphine or a placebo pellet for 6 or 24 hr. Naive animals were also implanted with either a morphine or a placebo pellet for the same period of time, to serve as controls.

Acute effects of morphine sulfate administration on SGOT and SGPT activities. Three groups of mice with eight in each group were given morphine sulfate, 10, 20 and 40 mg/kg, s.c., for 15 hr respectively. The control animals were given saline. Both SGOT and SGPT activities were determined as mentioned above.

Effects of intracerebroventricular (i.c.v.) injection of morphine on SGOT and SGPT activities. Three groups of mice with eight in each group were injected with saline and morphine sulfate, 10 and 20 μg/mouse, i.c.v., respectively, according to the method of Harris *et al.* [17]. The total volume injected was 10 μl. Both enzyme activities were determined 15 hr after morphine administration.

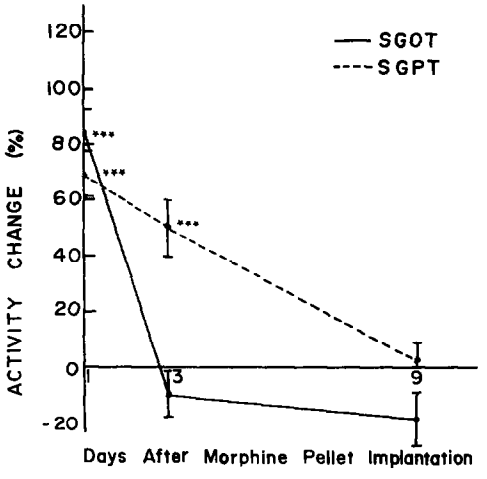

Fig. 1. Effects of continuous administration of morphine on SGOT and SGPT activities. Mice were implanted with a morphine pellet for 1 or 3 days. In mice receiving prolonged morphine pellet implantation, second and third pellets were implanted at 3-day intervals for a total of 9 days. The bracketed lines indicate ± S.E.M. of 3–5 replicates. Eight mice were used in each group. A triple asterisk (***) indicates $P < 0.001$.

RESULTS

Effects of continuous morphine administration on SGOT and SGPT activities. Continuous administration of morphine by pellet implantation caused an elevation of both SGOT and SGPT activities in a short time period. This phenomenon disappeared upon long-term pellet implantation. As shown in Fig. 1, there was a 2-fold increase in both SGOT and SGPT activities in mice which have been implanted with a morphine pellet for 1 day.SGOT activity returned to normal within 3 days. However, SGPT activity was still elevated significantly at 72 hr after morphine pellet implantation. In mice implanted with three morphine pellets for 9 days with each pellet implanted at 3-day intervals, both SGOT and SGPT activities were the same as placebo control levels.

In order to exclude the possibility that changes in SGOT and SGPT activities in morphine pellet-implanted animals may be due to altered eating habits (i.e. reduced food consumption), a further control experiment was carried out. In mice deprived of food and water for 24 hr, both SGOT and SGPT activities in both placebo and morphine pellet-implanted animals were not significantly different from enzyme levels in animals provided food and water *ad lib.*

The elevated SGOT and SGPT activities induced after 24 hr of morphine pellet implantation disappeared rapidly within 24 hr after the removal of the morphine pellet (Fig. 2). SGOT and SGPT activities returned to normal within 3 and 6 days, respectively, after the removal of the morphine pellet.

The elevations of SGOT and SGPT activities caused by morphine pellet implantation were attenuated significantly by concurrent administration of naloxone. As shown in Fig. 3, both SGOT and SGPT activities were elevated markedly at 12 and 24 hr after morphine pellet implantation. However, in mice concurrently given naloxone and implanted with a morphine pellet both

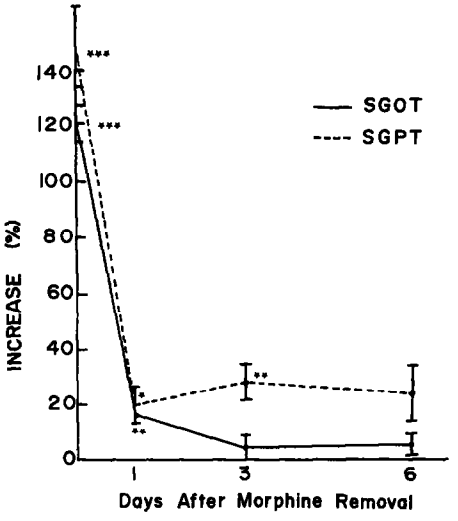

Fig. 2. Effects of abrupt withdrawal of a morphine pellet on SGOT and SGPT activities. Mice (eight in each group) were implanted with a morphine pellet for 24 hr. SGOT and SGPT activities were determined at different times after pellet removal. The bracketed lines indicate ± S.E.M. The single, double and triple asterisks (*, ** and ***) denote $P < 0.05$, 0.01 and 0.001 respectively.

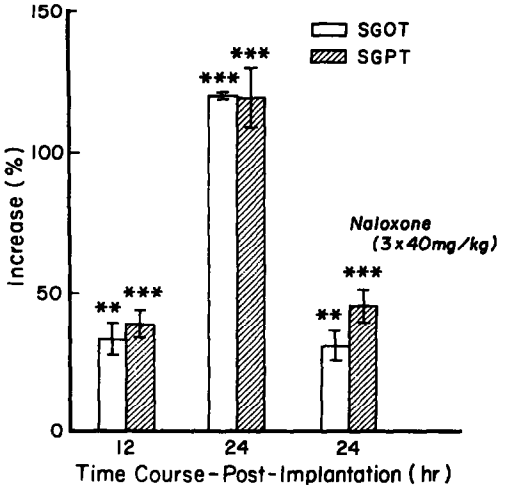

Fig 3. Effects of concurrent administration of naloxone on morphine-induced elevation of SGOT and SGPT activities. Two groups of eight mice were implanted with a morphine pellet for 12 and 24 hr respectively. The third group of mice was implanted with a morphine pellet for 24 hr with three concurrent administrations of naloxone hydrochloride, 40 mg/kg, s.c., at 8-hr intervals.

SGOT and SGPT activities were suppressed markedly.

Effects of acute morphine administration on SGOT and SGPT activities. SGOT and SGPT activities were also affected by acute injection of morphine. As shown in Fig. 4, in mice receiving morphine sulfate ranging from 10 to 40 mg/kg, s.c., both SGOT and SGPT activities were elevated significantly 15 hr after the administration of morphine.

Effects of hypophysectomy and adrenalectomy on SGOT and SGPT activities. The elevations of SGOT

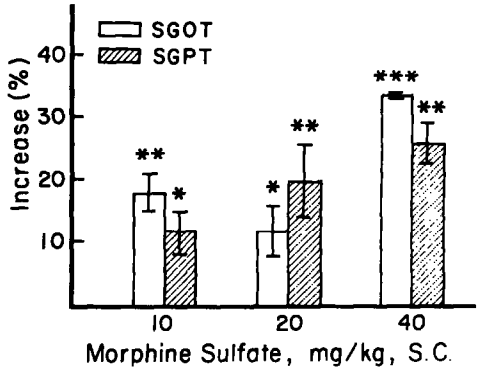

Fig. 4. Effects of acute s.c. administration of morphine sulfate on SGOT and SGPT activities. Mice were injected with morphine sulfate 10, 20 and 40 mg/kg, s.c., respectively. The control group received saline. SGOT and SGPT activities were assayed 15 hr after the drug administration. Eight mice were killed for each group. The bracketed vertical lines represent ± S.E.M.

and SGPT activities caused by morphine pellet implantation were prevented completely by hypophysectomy but not by adrenalectomy. In mice implanted with a morphine pellet for 6 hr, both SGOT and SGPT activities were elevated significantly in normal and adrenalectomized animals (Fig. 5). In contrast, SGOT and SGPT activities were not elevated in hypophysectomized mice. However, adrenalectomy partially blocked the elevation of SGOT and SGPT activities at 24 hr after morphine pellet implantation.

Effects of intraventricular injection of morphine on SGOT and SGPT activities. The administration of morphine sulfate by intraventricular injection also caused elevation of both SGOT and SGPT activities. As shown in Fig. 6, morphine sulfate, 20 μg/mouse, i.c.v., significantly elevated SGOT and SGPT activities.

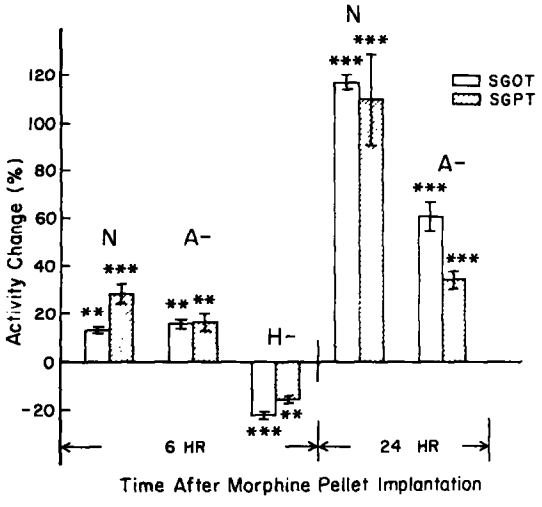

Fig. 5. Effects of hypophysectomy and adrenalectomy on morphine induced elevation of SGOT and SGPT activities. Hypophysectomized and adrenalectomized mice were implanted with either morphine or a placebo pellet for 6 or 24 hr. Naive animals were also implanted in the same fashion to serve as controls. Eight mice were used in each group. A^-, H^- and N denote adrenalectomized, hypophysectomized and naive animals. The bracketed vertical lines represent ± S.E.M.

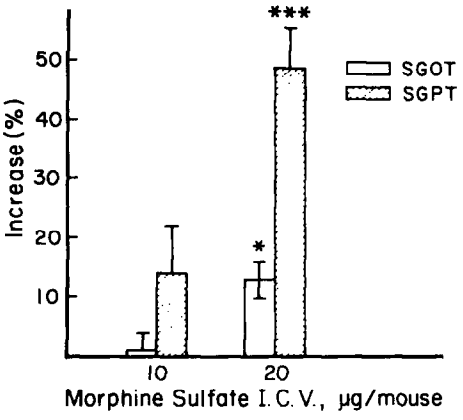

Fig. 6. Effects of acute intraventricular administration of morphine sulfate on SGOT and SGPT activities. Two groups of eight mice received morphine sulfate, i.c.v., 10 and 20 μg/mouse, respectively. Mice were killed 15 hr after drug administration. The control group received 10 μl saline, i.c.v. The bracketed vertical lines represent ± S.E.M. Asterisks refer to degree of significance. * = P 0.05 and *** = P 0.001.

DISCUSSION

The data presented here demonstrate that, when mice are continuously exposed to morphine by pellet implantation, both SGOT and SGPT activities are elevated significantly during the initial morphine implantation. The elevations of both SGOT and SGPT activities were observed only within 24 and 72 hr of the initial period of morphine pellet implantation respectively. In contrast, both SGOT and SGPT activities did not show significant elevation in mice which had been implanted with three morphine pellet for 9 days. The elevation of both SGOT and SGPT activities was partially attenuated by concurrent administration of naloxone. To insure that the elevation in SGOT and SGPT activities was indeed due to morphine effects instead of the drastic conditions of continuous pellet implantation, the effect of an acute single injection of morphine was also investigated. Although the magnitude of elevation of SGOT and SGPT activities was relatively smaller, the results obtained from a single injection of morphine sulfate substantiate the data obtained by continuous morphine pellet implantation.

The present studies also support our previous morphological and biochemical findings that administration of morphine by pellet implantation has a definite effect on liver function. Our previous ultrastructural studies showed that the hepatocytes of animals exposed to morphine pellets were filled with lipid droplets [8, 9]. Our biochemical studies also showed that morphine pellet implantation inhibited hepatic drug-metabolizing enzymes [11]. It also has been demonstrated in our laboratory that continuous morphine treatment results in a marked decrease in biliary excretion of endogenously formed metabolites of methadone in isolated perfused liver [18]. Our recent study on the increased rate of mortality by carbon tetrachloride during morphine pellet implantation also revealed that the enhancement of mortality rates by carbon tetrachloride was manifested only during the initial period of morphine pellet implantation [12].

Abnormalities of liver function are extremely common in narcotic addicts and occur frequently in the absence of signs, symptoms or a history of liver disease [3]. In a detailed study by Gorodetzky *et al.* [4], it was concluded that serum hepatitis of chronic narcotic addicts was caused by contamination from the dirty needles. The recent studies of Rosenthal [5] on comparisons of hepatic function among intravenous users, smokers and sniffers of heroin have also provided additional evidence that liver disease of narcotic addicts can be due to the injection of foreign materials rather than to opiates *per se*. However, the high incidence of transaminase abnormalities in patients who denied ever using intravenous drugs makes it impossible to exclude fully toxic effects of opiates. One of the main difficulties is the reliability of human subjects. When the samples were obtained from the narcotic addicts, there was little reliable information on history of drug taking, route of administration and duration of drug taking.

In our present studies, we have systematically assessed both acute and chronic administration of morphine on both SGOT and SGPT activities. The results demonstrate clearly that, while serum hepatitis of chronic narcotic addicts may be caused largely by contamination of hypodermic needle, the initial administration of morphine does indeed contribute to the abnormality of transaminase activity. It is reasonable to assume that the initial effect of morphine on the liver may result in an increased susceptibility to hepatic disorders.

The exact mechanism by which morphine affects hepatic function remains to be elucidated. However, the evidence from the present study suggests that it may be mediated through the central nervous system instead of by direct effects on the liver. Our results indicate that not only s.c. administration of morphine but i.c.v. administration as well causes elevation of both SGOT and SGPT activities. Our studies further indicate the involvement of the pituitary-adrenal gland. Pituitary-adrenal activation by morphine has been known for a long time. Adrenal ascorbic acid depletion was found in morphine-treated normal and adrenalectomized rats but not in hypophysectomized rats [19]. Single doses of the narcotic stimulated adrenal cortical response via the hypersecretion of ACTH, whereas prolonged administration produced depression of the basal levels of corticosteroid secretion [20]. An initial increase of corticosteroid urinary excretion in rats treated with morphine was followed by a decreased steroid excretion with subsequent narcotic administration [21]. Further studies on narcotic specificity and site of action are in progress.

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