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BRIEF COMMUNICATION

Role of Endorphins in Benzodiazepine-Induced Hyperglycaemia in Mice

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NAJIM, R. A. AND K. M. CLOR. *Role of endorphins in benzodiazepine-induced hyperglycaemia in mice.* PHARMACOL BIOCHEM BEHAV 46(4) 995-997, 1993.—The possibility that benzodiazepine-induced hyperglycaemia is mediated through the release of endogenous endorphins was tested. The results show that naloxone, the opiate antagonist, potentiated clonazepam-induced hyperglycaemia. Treatment with increasing doses of morphine for six days, which induced tolerance to endorphins, did not affect clonazepam-induced hyperglycaemia. The results indicate that endorphins do not mediate benzodiazepine-induced hyperglycaemia.

Blood glucose Clonazepam Morphine Naloxone

BENZODIAZEPINES are known to increase blood glucose in humans (10), in rats (5), and in mice (6,7), but the mechanism by which they do this is not yet established.

Benzodiazepines have been known to produce dose-dependent alterations of β-endorphine release (3,8). It has also been reported that the potent opiate antagonist naloxone blocked the behavioral effects of benzodiazepines (4,13). Since opioids were demonstrated to be involved in glucoregulation (1), this work was designed to test the possibility that benzodiazepine-induced hyperglycaemia is mediated through the opioid system.

MATERIALS AND METHODS

Animals

Male Swiss albino mice obtained from the breeding colony at the Baghdad College of Medicine and weighing between 20-25 g were used. Animals were housed in groups of five to six and had free access to a pellet diet and tap water. On the day of the experiment, food was withheld 2 h prior to drug administration.

Naloxone-Clonazepam Study

Naloxone (1 or 4 mg/kg) or the vehicle were injected SC. Fifteen minutes later, clonazepam or the vehicle were injected IP. Thirty minutes later, the mice were decapitated and trunk blood was collected for determination of blood glucose by the glucose oxidase-peroxidase method (12).

Morphine-Clonazepam Study

Tolerance to morphine was established by daily SC injection of 10, 10, 20, 20, 40, and 40 mg/kg of morphine for six successive days. Control animals received equal volumes of vehicle SC daily for six days. Twenty-four hours after the last injection of morphine, animals received either clonazepam (5 mg/kg IP) or an equal volume of vehicle. Mice were decapitated 30 min later and blood glucose was determined.

Drugs

Clonazepam supplied by Hoffmann-La Roche (Nutley, NJ) was suspended in normal saline to which Tween 80 was added (two drops of Tween 80 in 10 ml of normal saline). The dose was chosen on the basis of previous studies done in our laboratory which indicate that this dose induces a maximal hyperglycaemic effect (7). Naloxone ampoules (Dupont Pharmaceuticals) and morphine sulphate ampoules (Evans, Liverpool, England) were used in the study. The doses for naloxone were chosen on the basis of our pilot study as well as previous published reports indicating that these doses blocked some of the benzodiazepine effects (4,13).

All drugs were freshly diluted before administration. Drug

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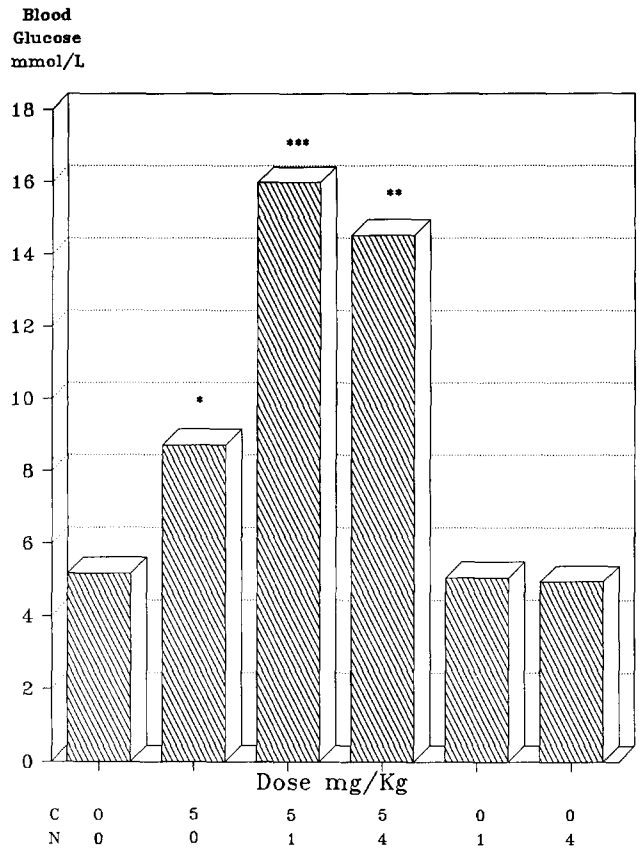

FIG. 1.

concentrations were adjusted so that each mouse received no more than 0.01 ml/g body weight. All experiments were performed between 1000 and 1100 to exclude the effect of circadian rhythm on the results.

Statistics

Data from each study were analyzed using a one-way analysis of variance (ANOVA) for independent groups and also compared by Student's t test. A level of $p < 0.05$ was considered to be minimum for statistical significance.

RESULTS

Naloxone-Clonazepam Study

Clonazepam induced a significant hyperglycaemia when administered following a SC injection of saline. When naloxone was administered SC in doses of 1 or 4 mg/kg before clonazepam, there was a significant increase in blood glucose, $F = 17.3995$, $p < 0.005$, even compared to the saline-clonazepam-treated group. Naloxone alone failed to have a significant effect on blood glucose (Fig. 1).

Morphine-Clonazepam Study

Chronic administration of saline for six days did not attenuate clonazepam-induced hyperglycaemia, nor did daily administration of morphine affect clonazepam-induced hyperglycaemia, $F = 6.505$, $p < 0.003$ (Fig. 2).

DISCUSSION

The mechanisms involved in benzodiazepine-induced hyperglycaemia are still unclear. In this study, we tested the possibility that benzodiazepine-induced hyperglycaemia might be mediated through release of endorphins. The results of the naloxone study clearly indicate that not only did naloxone fail to block the hyperglycaemic effect of clonazepam, but it potentiated it, and more markedly in the lower dosage used (1 mg/kg).

Morphine was administered in increasing doses for six days. This treatment was shown to induce morphine tolerance, which lessens the degree of responsiveness of opiate receptors to the effect of administered endorphins (2,11,14). However, this treatment did not affect clonazepam-induced hyperglycaemia either.

Therefore, the results from the two studies indicate that opiates do not mediate benzodiazepine-induced hyperglycaemia. The results showed that naloxone potentiated the hyperglycaemic effect of clonazepam. Naloxone has been reported to reduce insulin secretion from isolated pancreatic cells (9). This effect may explain the potentiation obtained in this study.

The results of the present study indicate that some endogenous opiate systems may not be involved in the hyperglycaemic effect of benzodiazepines. Other mechanisms, including a direct effect of benzodiazepines on glucoregulatory centers in the brain, or even the pancreas, may have to be investigated.

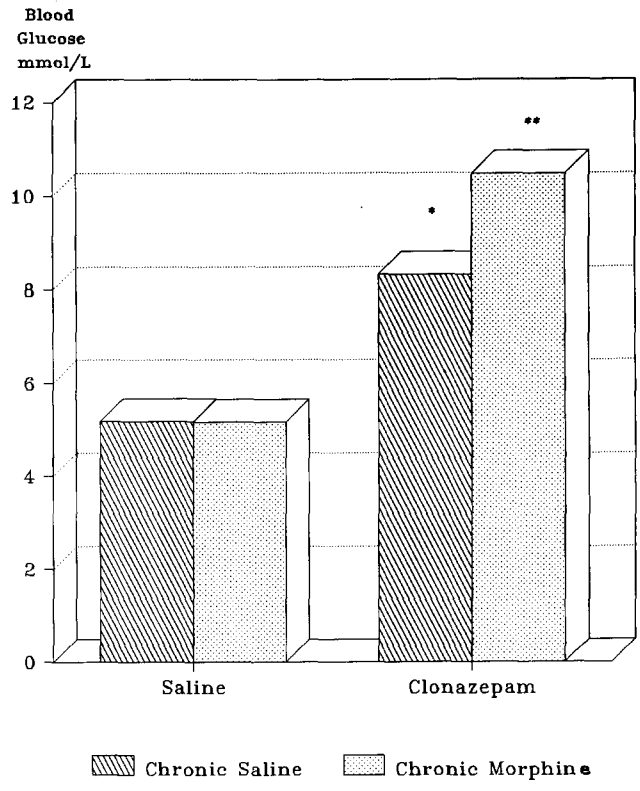

FIG. 2.

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