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Evidence of Hyperglycemic Hyperalgesia by Quinpirole

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ROANE, D. S. AND D. PAUL. *Evidence of hyperglycemic hyperalgesia by quinpirole*. PHARMACOL BIOCHEM BEHAV **41**(1) 65–67, 1992.—Male albino rats were tested for antinociception following injections (IP) with saline, quinpirole (Quin) (1 mg/kg), morphine sulfate (M.S.) (5 mg/kg), or both Quin and M.S. (1 mg/kg and 5 mg/kg, respectively). Quin reduced and M.S. increased tail-flick latency as compared to controls. Tail-flick latencies of the animals injected with both drugs were significantly reduced as compared M.S. alone. Quin increased blood glucose levels by 96 percent, as compared to saline controls. In competitive binding studies Quin displaced ^{3}H-DAGO (IC_{50} = 29.8 μM). CD-1 mice demonstrated a naloxone-reversible analgesia following ICV Quin (100 μg). These data are consistent with the hypothesis that the hyperglycemic effects of Quin attenuate M.S. analgesia while the antinociceptive effects of Quin may be mediated through opioid receptors.

Antinociception Analgesia Hyperglycemia Quinpirole D-2 agonist Glucose Opioid receptor

THE elevation of plasma glucose, produced either by streptozotocin pretreatment (6, 9, 10) or by simple IP injection of sugars (4) attenuates the antinociceptive potency of morphine (M.S.). The mechanism of action of this phenomenon is unknown, though there appears to be some involvement of CNS cellular energetics, including ATP synthesis (10) and an ATP-sensitive K^+ channel (5). It has been reported that the dopamine-2 receptor agonist, quinpirole (Quin) elevates plasma and brain glucose via a central D-2 mechanism when the drug is administered in small doses, peripherally (8). This raises the possibility that Quin might interfere with M.S.-mediated antinociception via a hyperglycemic effect, in spite of the fact that Quin, at higher doses, possesses analgesic properties (7).

METHOD

Male Sprague-Dawley rats, 250–350 g, were housed under standard conditions in individual cages on a 12-h light cycle (lights on at 0700 h) with ad lib access to Purina Rat chow and water. All procedures were begun at 0800 and completed by 1100 h.

Nociceptive thresholds were assessed by the tail-flick method of D'Amour and Smith (1) with the stimulus intensity set to elicit a response from control animals at 5.5 ± 0.5 s. All animals were handled daily for three days and were subjected to the tail-flick procedure on two consecutive days prior to drug testing.

Morphine sulfate (M.S.) (NIDA Drug Supply System, Rockville) and quinpirole HCl (Quin) (RBI, Natick, MA) were placed in solution at concentrations of 5 and 1 mg/ml, respectively. Drugs were administered 1 ml/kg body weight, IP, 30 minutes prior to tail-flick testing. Control animals received saline in equivalent volumes.

Blood samples for glucose analysis were taken from the tails of the animals immediately following the nociceptive tests. Plasma glucose was assayed by glucose oxidase method adapted for a YSI Model 23A Glucose Analyzer (Yellow Springs, OH).

Rat brain membranes for mu opioid receptor binding were prepared by homogenizing tissue in 50 volumes of 0.32 M sucrose–50 mM Hepes buffer, pH 7.4 in a glass vessel with a teflon pestle. The resulting suspension was centrifuged at 1000 × g for 8 min. The supernatant was decanted, centrifuged and washed three times at 30,000 × g in 50 mM Hepes HCl, pH 7.4. The resulting pellet was resuspended in 50 volumes of Hepes buffer.

For binding analysis, 500 μl of membranes were used to make up 1 ml final volumes containing 4.8 nM ^{3}H-DAGO ([D-Ala^2, N-Me-Phe^4, Gly-ol^5] enkephalin) (NEN, Boston, MA) with Quin concentrations ranging from 1×10^{-11} M to 1.22×10^{-04} M. Nonspecific binding was determined by ^{3}H-DAGO binding in the presence of 10 μM cold DAGO and total binding was determined by the binding of 4.8 nM ^{3}H-DAGO in the presence of no competitors. Incubations were carried out at 25°C for 90 min. Samples were poured over Whatman GFB filters on a vacuum manifold and washed with 3 × 5 ml of ice-cold buffer. Filters were dried and counted by standard scintillation techniques. Displacement curves and estimation of the IC_{50} of Quin for the mu receptor were obtained by use of the ALLFIT program with the "a" and "d" parameters restricted to values of 100 and 0, respectively (2).

The mouse Quin-analgesia studies used CD-1 mice maintained under standard conditions. The D'Amour and Smith tail-flick method was used to establish baseline latencies with the mean of two trails in the range of 3–4 s. Posttreatment tail-flick latencies were determined 20 min after central injections. Mice doubling their baseline latency were considered analgesic. A maximal latency of 12 s was used to minimize tissue damage.

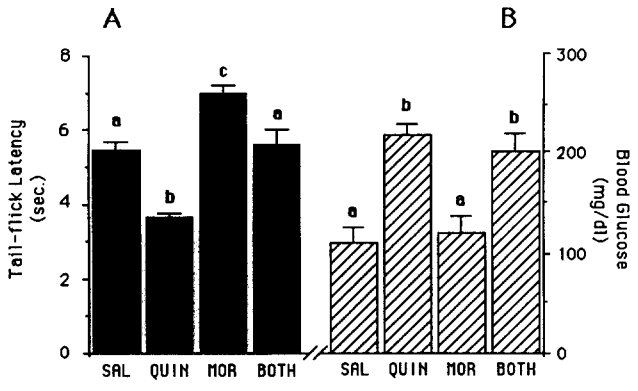

FIG. 1. (A) The tail-flick latencies (s) of S.D. rats 30 min after the injection of saline, quinpirole (1 mg/kg), morphine sulfate (5 mg/kg) or both quinpirole and morphine, simultaneously. Columns headed by differing letters are statistically significant (n = 9/group). (B) The plasma glucose values (mg/dl) from the animals taken immediately following the tail-flick procedure. Again, differing letters denote statistical significance (n = 9/group).

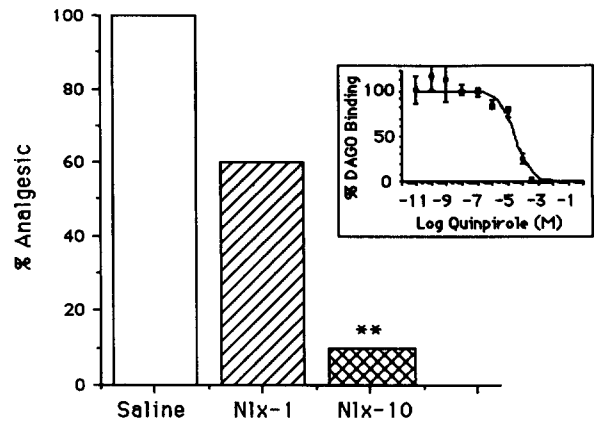

FIG. 2. This figure shows the dose-dependent inhibition of quinpirole analgesia by naloxone. Groups of mice (n = 10) were treated (SC) with saline, 1.0 mg/kg naloxone, or 10.0 mg/kg naloxone 30 min before tail-flick testing. All mice received Quinpirole (100 μg, ICV) 20 min before testing. Quinpirole analgesia was significantly attenuated by 10.0 mg/kg naloxone (Fisher Exact Test, $p<0.01$). The inset shows the ability of quinpirole to displace the mu opioid receptor ligand ^{3}H-DAGO, IC_{50} = 29.8 μM.

Introcerebroventricular (ICV) injections were made in a volume of 10 μl under light halothane anesthesia using a Hamilton 10 μl syringe fitted to a 30-gauge needle with PE10 tubing. Injections were administered 2 mm caudal and 2 mm lateral to bregma at a depth of 3 mm (3). Naloxone or saline was administered subcutaneously in a volume of 0.1 ml/kg, 30 min before testing. Statistical significance for naloxone inhibition of analgesia was assessed by the Fisher Exact test.

All other statistical analyses were performed by Least Square Means with the Bonferroni correction in cases of multiple comparisons.

RESULTS

The effects of Quin, M.S., and the coadministration of both drugs on nociception, in rats, as measured by the tail-flick test are shown is in Fig. 1A. Compared to the saline-injected animal' tail-flick latency (5.44 ± 0.23 s), Quin significantly decreased (3.65 ± 0.09 s, $p<0.0001$) and M.S. significantly increased (6.98 ± 0.23, $p<0.0005$) the response time. Coadministration of Quin and M.S resulted in latencies significantly lower than M.S. alone (5.58 ± 0.42, $p<0.0014$).

Plasma glucose levels of the saline-controls averaged 110.67 ± 15.26 mg/dl. M.S. caused a slight and statistically insignificant increase in plasma glucose (119.22 ± 17.34), while Quin and Quin plus M.S. values were statistically increased, (216.67 ± 11.42, $p<0.0011$) and (199.89 ± 18.8, $p<0.0004$), respectively, compared to the saline controls (Fig. 1B).

The ICV injection of 100 μg Quin produced analgesia in 100 percent of the mice tested. Naloxone dose-dependently attenuated quinpirole analgesia ($p<0.01$ at the 10 mg/kg dose, Fig. 2).

Results of the binding studies provided an estimate of the IC_{50} of Quin against ^{3}H-DAGO, $2.98 \times 10^{-5} \pm 0.245 \times 10^{-5}$ M (Fig. 2, inset).

DISCUSSION

The results from these experiments indicate that peripherally administered Quin, in relatively low doses, decreases nociceptive thresholds as measured by the tail-flick test and attenuates the analgesic potency of M.S. Simultaneously, with the changes in nociception, Quin produces a distinct increase in blood glucose. We believe that the hyperglycemia is responsible for the hyperalgesic effects of Quin. There are numerous previous reports in the literature showing that elevations of blood glucose, either by diabetes induction or by the direct injection of sugars, results in diminished opiate analgesia (4, 6, 9, 10). At least a portion of opioid-mediated analgesia occurs as the result of opioid receptor linkages to the opening of an ATP-sensitive K^+ channel (5). We hypothesize that the phenomenon of hyperglycemic hyperalgesia occurs in the following manner: as blood glucose rises, brain glucose rises, resulting in increased neuronal glucose uptake and ATP synthesis. The increase in intracellular ATP opposes the agonist activity of the opioid by closing the ATP-sensitive K^+ channel. This hypothesis is supported by previous work that has shown that glucose and Krebs' cycle intermediates attenuate morphine analgesia, while inhibitors of ATP synthesis enhance morphine analgesia (10), and an ATP-sensitive K^+ channel blocker antagonizes morphine analgesia (5).

Rooney and Sewell (7) demonstrated that Quin, administered in 100 μg doses, ICV, in mice, produced a profound analgesia. Our study confirms this finding. However, 100 μg of Quin in the brain of a mice will likely produce brain concentrations of the drug in the micromolar range, i.e., if a volume of distribution of 4 ml is assumed, the brain concentrations would be 98 μM. We found that Quin displaced the mu-opioid receptor selective compound, DAGO, with an IC_{50} at approximately 29 μM. With a Quin brain concentration of 98 μM more than 70 percent of the mu receptors would be occupied. If Quin possesses some level of intrinsic activity at the mu receptor, 70 percent receptor occupancy would provide a profound analgesia. The dose-dependent naloxone-reversibility of quinpirole-mediated analgesia also implies opioid agonist activity. We believe these findings intimate, but by no means prove, that the analgesic effects of Quin may be mediated through mu opioid receptors. We believe numerous other interpretations of this data are possible due to the fact that we did not measure Quin displacement of ^{3}H-DAGO in mouse brain, Quin-induced hyperglycemia in mice or ICV Quin analgesia in rats.

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