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RAPID COMMUNICATION

Analgesic Actions of Local Anesthetics and Cobalt Chloride in the Rat Brain Stem

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HAMANN, S. R., J. R. HOLTMAN AND W. R. MARTIN. *Analgesic actions of local anesthetics and cobalt chloride in the rat brain stem.* PHARMACOL BIOCHEM BEHAV 43(3) 925-927, 1992.—A low-intensity thermally evoked tail avoidance reflex (LITETAR) was used to study changes in nociceptive response produced by local anesthetics and cobalt chloride microinjected into the dorsal posterior mesencephalic tegmentum (DPMT) of conscious rats. Dose-related prolongation of the LITETAR (e.g., analgesia) was observed when lidocaine, cocaine, and bupivacaine were administered into the DPMT. Analgesic actions were also demonstrated when cobalt chloride was microinjected into the DPMT. The analgesic actions of these different neuronal suppressants provide support for the hypothesis that there exists tonic activity of hyperalgesic processes in the rat brain stem.

Analgesia Hyperalgesia Opioid Tonic activity Brain stem Nociception

PREVIOUS studies have shown fourth-ventricle opioid and nicotinic hyperalgesic processes in the dog and rat (10,14, 16,23). The pharmacology of hyperalgesia in the region of the fourth ventricle involves several mechanisms including μ-ergic (morphine) and κ-ergic (ethylketazocine [EKC] and U-50,488H) opioid and possibly two nicotinic cholinergic mechanisms (7,14–16). These fourth-ventricle hyperalgesic processes are thought to exhibit tonic activity because either naltrexone or mecamylamine produce dose-related analgesia (10,16). Recent investigations have demonstrated opioid and nicotinic hyperalgesic processes from the midmedulla to the posterior dorsal mesencephalic tegmentum (DPMT) and that the DPMT is the most active site thus far tested (9). Naltrexone and mecamylamine produce dose-related analgesia, suggesting the presence of tonic hyperalgesic activity in these regions of the rat brain stem (9).

The purpose of this article is to further test the hypothesis that agents that suppress neuronal function will also produce analgesia when administered into the DPMT.

METHOD

Female Sprague-Dawley rats (250–300 g) were implanted with stainless steel 22-ga guide cannulae directed toward a position within the DPMT [AP 0.2, L 0.0, V +3.0, interaural (17)] under ketamine (100 mg/kg)-acepromazine (1 mg/kg) anesthesia. Animals were allowed to recover 1 week thereafter. At 2-day intervals, graded doses of drugs were microinjected (0.5 μl) using a 28-ga chemotrode connected to a 1-μl Hamilton syringe with polyethylene tubing. Drug-induced changes in response to nociceptive stimulus were assessed using a low-intensity thermally evoked tail avoidance reflex (LITETAR) (16). The stimulus strength was determined using thermopile equilibrium conductance values at the beginning and throughout each experiment. The LITETAR was evoked with a heat stimulus intensity sufficient to give a mean control latency of about 20 s. The cutoff time for the LITETAR was 40 s. The LITETAR has been shown to be more sensitive than higher-intensity tail flick assays in detecting the analgesic actions of intraperitoneal opioid agonists including morphine, EKC, and U-50,488 (9,15,16). In addition, the LITETAR detects the analgesic actions of naltrexone and mecamylamine (9,15,16).

Experiments were conducted using complete crossover design. Each animal received several doses of each drug and vehicle. Two predrug LITETAR determinations were made at 10-min intervals. Mean predrug latencies were subtracted from observations made at 5, 10, 15, 20, 30, 45, and 60 min after microinjection. LITETAR determinations were also made at 75 and 90 min following cobalt Cl ($CoCl_2 \cdot 6H_2O$)

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microinjection. These values were used for determining the time action curves from which area under the curve (AUC) was calculated using the trapezoidal rule for construction of dose-response lines. The vehicle areas were subtracted from the drug treatment area. Analyses of variance (ANOVAs) were performed for the 0- to 30-min portions of the dose-response data. The total variance was partitioned into between-rats, -dose, and -error variance. The between-dose variance was further partitioned into regression variance and variance due to deviation from linearity (3). Drug-induced differences from predrug latencies were determined using Student's *t*-test.

Lidocaine, cocaine, and $CoCl_2$ were dissolved in 0.9% NaCl. The doses of lidocaine studied were 2.5, 10, and 20 μg. The doses of cocaine studied were 5.0, 22.5, and 45.0 μg. Bupivacaine was dissolved in dimethyl sulfoxide and only two doses (30 and 75 μg) were studied. Each drug or vehicle was microinjected in a volume of 0.5 μl. Drug doses presented are the free base. The dose of $CoCl_2$ (12 μg) is expressed as the salt. At the end of the experiments, two to four rats from each experimental group were microinjected with 0.5 μl methylene blue (0.5%) through the chemotrode into the DPMT. Animals were killed by an overdose of pentobarbital (65 mg/kg). Brains were removed following decapitations and fixed in formalin (10%). Brains were subsequently examined 2-3 days later to determine the sites of staining. All drugs were purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Hyperalgesia and analgesia will be utilized to indicate shortening and prolongation of the LITETAR, respectively. Dose-response curves for the local anesthetics are presented in Fig. 1. Because of low solubility, only a small and modestly analgesic dose of bupivacaine could be administered. Lidocaine appeared to be the most potent of the local anesthetics in producing analgesia. As indicated in Fig. 1, significant regression of between-dose variance was determined for both

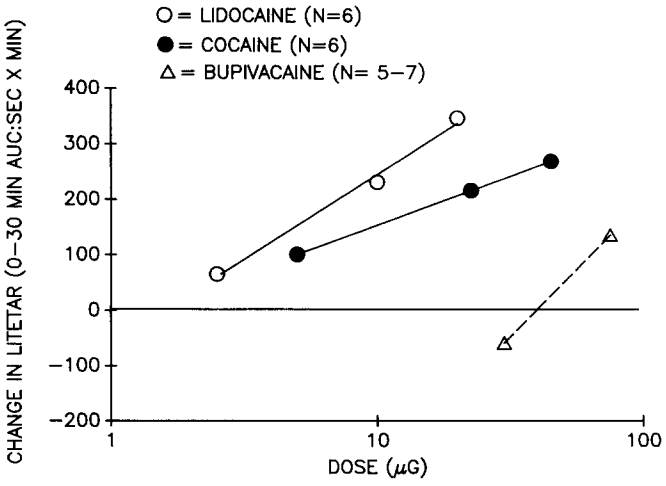

FIG. 1. Dose-response curves for lidocaine, cocaine, and bupivacaine when graded doses were administered into the DPMT. Solid lines represent those portions of the dose-response lines that exhibited significant regression. Data previously reported for lidocaine are included (6). Data points represent the mean change in latency for the number of rats studied.

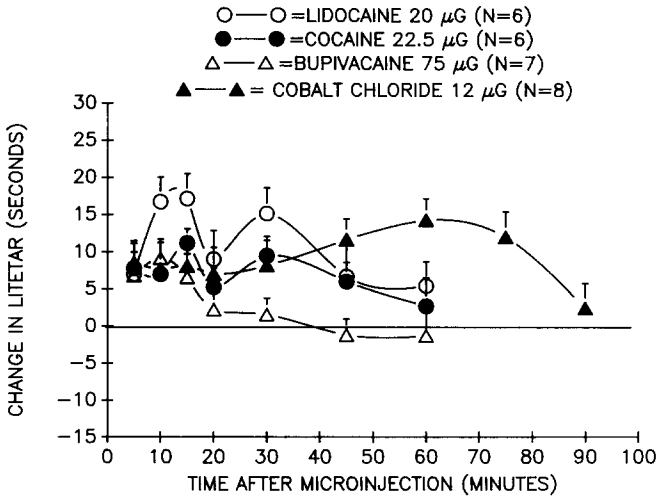

FIG. 2. Example time-action curves for analgesic actions of lidocaine, cocaine, bupivacaine, and cobalt Cl after microinjection into the DPMT. Data points represent mean ± SE for the number of rats indicated. Each drug shown produced significant ($p < 0.05$ to $p < 0.001$) effects within 5–20 min following microinjection.

lidocaine and cocaine without significant deviation from regression. We were unable to study repeated doses of cobalt Cl because the latency of the LITETAR was prolonged or absent during the week following microinjection.

Figure 2 shows example time-action curves for lidocaine, cocaine, bupivacaine, and cobalt Cl following microinjection into the DPMT. All drugs produced a rapid onset of analgesia. Bupivacaine had a shorter duration of analgesic action (15–20 min) than cocaine or lidocaine (45–60 min). The analgesic actions of $CoCl_2$ persisted for 75–90 min.

Sites of microinjection in the DPMT were established using methylene blue in two to four rats within each series of drug experiments. The dye was distributed within 1–1.5 mm surrounding the intended site of administration, with the darkest staining present within approximately a 1-mm radius. There was slight staining observed along the tract of the guide cannulae, probably resulting from removal procedures upon decapitation. In a few specimens, there was faint staining near the rostral extent of the fourth ventricle.

DISCUSSION

Previous studies have demonstrated the analgesic (antihyperalgesic) actions of different pharmacological agents including opioid and nicotinic antagonists (6,10), local anesthetics (13), and dynorphin A(1-13) antiserum (8) after microinjection into a hyperalgesic region within the DPMT. The results of the present studies provide further support for the hypothesis that there may be tonic activity of brain stem hyperalgesic processes and that reduction of this tone results in analgesia. Lidocaine, cocaine, and bupivacaine produced dose-related prolongation of the LITETAR. Cocaine was somewhat less potent than lidocaine in producing analgesia as it is in producing nerve block (6). Bupivacaine was less potent in this regard and had a shorter duration of action. It is not known to what extent these differences may be due to solubility differences or more rapid uptake by blood and redistribution to other potential sites of action. Local anesthetics have been employed in several laboratories to produce reversible neuronal block

after microinjection into the brain (2,4,5,18–22). For the most part, the time-action data for lidocaine's analgesic actions shown in Fig. 2 are comparable to the duration of lidocaine blockade of 30–60 min demonstrated in the ventromedial medulla (21). These same animal studies showed that lidocaine may produce partial block of neuronal conductance with changing distribution for longer than 2 h (21). The contributions of partial depolarization or conduction block to the complex time course of analgesic actions observed for local anesthetics in the DPMT (Fig. 2) have not been explored.

The analgesic actions of $CoCl_2$ when microinjected into the DPMT support the presence of tonic hyperalgesic activity. Cobalt Cl is a neuronal inhibitor that appears to have selectivity for synaptic transmission (11,12). $CoCl_2$ has been shown to reversibly block transmission for 60 min when microinjected in 25 nl in concentrations similar to those used in this study (1). $CoCl_2$ has also been shown to produce permanent neuronal damage (12).

In summary, these studies confirm and extend previous reports that agents that suppress neuronal activity produce analgesia when microinjected into a region of the DPMT shown to contain opioid and nicotinic hyperalgesic mechanisms. These findings are consistent with the hypothesis that suppression of tonic hyperalgesic processes in the rat brain stem results in analgesia.

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