Intracerebroventricular Drug Administration in Pigeons

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MACOL BIOCHEM BEHAV 23(5) 731-736, 1985.-For many procedures used in behavioral pharmacology, the intracerebroventricular (ICV) route of drug administration is infrequently used due, in part, to the lack of a reliable technique for determining cannula patency in vivo. This study describes an in vivo technique for assessing ICV cannula patency in pigeons. The technique was applied in an experiment designed to evaluate several drugs, which are presumed to differ in the extent to which they enter the central nervous system, for their rate-suppressing effects in pigeons trained to peck a key on a fixed-ratio 20 schedule of food reinforcement. The opioid agonist morphine and antagonist quaternary naltrexone were 100 and 280 times more potent, respectively, in suppressing responding when administered ICV, as compared to systemic administration. Tertiary naltrexone was approximately equipotent as an antagonist of morphine's rate-suppressing effects when administered ICV or systemically. Quaternary naltrexone did not antagonize morphine by either route of administration. The utility of this in vivo cannula verification technique is discussed, as well as the limitations of comparisons between systemically-administered tertiary and quaternary derivatives.

SEVERAL strategies have been used for examining the importance of central and peripheral mechanisms of pharmacological effects in vivo. A marked difference in the potency of a compound when administered centrally and peripherally can indicate the relative importance of central and peripheral mechanisms. In rats, for example, the analgesic [3, 13, 14], discriminative [6, 18], rate-suppressant [2], and locomotor-increasing [1] effects of opioid agonists (e.g., morphine) occur with much smaller doses when these drugs are administered directly into the central nervous system (CNS) as compared to the doses needed to induce these effects after systemic administration. Thus, these effects of opioids are mediated predominantly by central mechanisms.

Comparisons of the behavioral effects of systemically-administered tertiary and quaternary derivatives of drugs have also been used as indices of central and peripheral mechanism. Quaternization of the opioid antagonist naltrexone, for example, does not markedly affect its ability to displace [3H]etorphine from rat brain membranes or its ability to antagonize morphine’s inhibitory effects on the guinea pig ileum [19]. However, in most, but not all [5, 12, 17] in vivo, experiments neither quaternary opioid antagonists [5, 16, 19] nor quaternary opioid agonists [11] mimic the actions of their respective tertiary forms when administered systemically. Given the similar spectrum of action of quaternary and tertiary naltrexone in vitro, and the marked differences in the effects of these compounds when administered systemically in vivo, it is generally assumed, for example, that the lack of activity displayed by quaternary antagonists in vivo is a direct result of their inability to enter the CNS [4]. These data have provided presumptive, although not conclusive, evidence that the mechanisms responsible for many of the actions of opioid agonists and antagonists are located within the CNS. More direct evidence can be obtained from procedures which examine the effects of tertiary and quaternary derivatives when given by various routes of administration (e.g., [5, 9]). To date, most experimental procedures which involve the administration of drugs directly into the CNS are of short duration, and cannula placement can only be determined with histological techniques upon termination of the experiment. The present study illustrates the long-term use of a cannula system in pigeons and the usefulness of a non-invasive radiographic verification procedure; relatively little is known about the pharmacological and behavioral significance of the blood-brain barrier (BBB) in the pigeon, despite the extensive use of this species within behavioral pharmacology. In addition, some results are described for compounds presumed to have different capacities for entering the CNS. The rate-suppressing effects of intracerebroventricular (ICV) and intramuscular (IM) morphine, naltrexone (tertiary), and naltrexone methobromide (quaternary naltrexone) were examined, as well as the effectiveness of ICV and IM naltrexone and quaternary naltrexone in antagonizing the rate-suppressing effects of IM morphine.

1Supported by USPHS Grant DA 00154. Portions of these data were presented at the 92nd Annual Convention of the American Psychological Association, Toronto, August, 1984 [8].
2Requests for reprints should be addressed to Charles P. France, Department of Pharmacology, 6322 Medical Science Building I, University of Michigan Medical School, Ann Arbor, MI 48109-0010.
Subjects

White Carneaux pigeons (Palmetto, Sumter, SC) weighing 401–770 g were reduced to 80% of their free-feeding weight. The reduced body weight was maintained by food earned during experimental sessions and supplemental feeding in the home cage (mixed grain and Purina Pigeon Checkers) where water and grit were freely available. Twenty-six pigeons have been cannulated to date for various experiments; the data in the present study were obtained with eleven pigeons.

Apparatus

Experimental sessions were conducted in a ventilated, sound-attenuated operant chamber measuring 36 cm high × 28 cm wide × 33 cm long. Three translucent response keys (2.4 cm diameter) were located on the inside of one wall, approximately 25 cm from the chamber floor. During periods of food availability, the center key was transilluminated green by a 7-W light located behind the key. Reinforcement consisted of a 4-sec access to mixed grain, made available via a hopper that pivoted into an opening in the wall, directly below the response key. During reinforcement the key light was off and a white light illuminated the hopper. A Texas Instruments 960A computer (Texas Instrument, Inc., Dallas, TX) and cumulative response recorders (Ralph Gerbrands Co., Arlington, MA), located in an adjacent room, were used for control of experimental events, data collection, and recording.

Surgery

Pigeons were anaesthetized with 2.5 ml/kg Chloropent (chloral hydrate and pentobarbital (Fort Dodge Laboratories, Inc., Fort Dodge, IA)) and 5.0 mg/kg ketamine, and a chronic, indwelling guide cannula (8.5 mm in length, 22 g stainless steel (Plastic Products, Inc.)) was surgically implanted using stereotaxic procedures and a Revzin adaptor [15]. An attempt was made to place the tip of the guide cannula directly into the lateral ventricle using the coordinates: 8.8 mm dorsal and 6.8 mm rostral from the interaural axis, and 1.5 mm lateral from the midline (Fig. 1). The cannula was permanently fixed with skull screws and Craniplastic.

Following surgery, and once every month thereafter, each bird was anaesthetized with 25.0 mg/kg ketamine and cannula patency was assessed by radiographs taken immediately after an ICV injection of 10–20 µl (6–12 mg) radio-opaque dye (Conray (Mallinckrodt, Inc., St. Louis, MO)). A 28 g dummy cannula (Plastic Products, Inc.) was inserted into the guide cannula, except during infusions.

Procedure

Pigeons were trained to respond on a single key by differential food reinforcement of successive approximations of the key peck. Experimental sessions were conducted on three consecutive days each week, and consisted of five discrete 15 minute trials. Each trial consisted of a ten minute pretreatment period, during which the chamber was dark and key pecks had no programmed consequence, and a five minute response period, during which reinforcement was available on a variable-interval 30 second (VI30") schedule of food delivery (i.e., the first response, after an average inter-
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FIG. 2. Radiographs taken prior to (left panel) or immediately after (center and right panels) an ICV injection of 10–20 µl radio-opaque dye. The pigeons shown in the center and right panels were judged to have a positive and a negative cannula placement, respectively.

MORPHINE  NALTREXONE  QUATERNARY NALTREXONE

FIG. 3. Dose-effect curves for the rate-suppressing effects of ICV (open symbols) and IM (closed symbols) morphine (left panel), tertiary naltrexone (middle panel), and quaternary naltrexone (right panel). Injections were made ten minutes prior to the first of five test trials, and each point represents the mean ± S.E.M. of four pigeons, for all five trials. Ordinate: mean response rate, expressed as a percentage of the saline control rate. Abscissa: dose in milligrams per kilogram body weight. Fixed doses were administered ICV (e.g., 100 µg), however for display purposes the fixed doses have been converted to mg/kg.

trexone methobromide (MRZ 2663BR, Dr. H. Merz, C. H. Boehringer Sohn, Ingelheim am Rhein, Federal Republic of Germany). Morphine and naltrexone were dissolved in sterile 0.9% saline for systemic administration, and in sterile water for central administration. Naltrexone methobromide was dissolved in sterile water.

RESULTS

Cannula Verification

Cannula placement was determined by x-rays in which the distribution of radio-opaque dye delineated the ventricular horn. Cannula placement was judged to be negative if the dye appeared to diffuse to areas other than the ventricular space or, more typically, if dye was concentrated only at the cannula tip (Fig. 2, right panel). Of 26 surgical attempts to date, 19 (73%) have resulted in successful placement of the guide cannula tip into the lateral ventricle, as determined by diffusion of the dye throughout the lateral ventricle (Fig. 2, center panel). Of 19 subjects judged initially to have positive cannula placement, four died from causes unrelated to cannulation, two lost their cannula within five months of surgery (apparently a result of the cannula being bumped or caught in the home cage), four were subsequently judged negative after 10.4±3.2 months (range=2.0–17.5), and nine pigeons are still positive after 9.7±1.1 months (range=4.1–17.0). Only data obtained from experiments which were both preceded and followed by a positive x-ray were included in the analysis.

Rate-Suppressing Effects of Morphine, Naltrexone, and Quaternary Naltrexone

Morphine dose-dependently suppressed food-reinforced responding when administered ICV or IM. A dose of 10.0 or 32.0 mg/kg morphine IM suppressed responding to a mean rate of less than 10% of the saline control rate over the five trial session, while a dose of 100.0 µg (0.21 mg/kg) ICV was required to produce a comparable suppression of rates (Fig. 3). Thus, morphine was 50–150 times more potent in suppressing behavior when administered ICV.

Quaternary naltrexone also suppressed responding by both routes of administration. Systemically, 100.0 mg/kg was the smallest dose of quaternary naltrexone that reliably suppressed response rates to less than 10% of the control rate (Fig. 3). Administration of 100.0 µg (0.206 mg/kg) quaternary
naltrexone ICV suppressed responding completely over the 1/4 hour test session and was accompanied by violent shaking and vomiting.

Tertiary naltrexone (IM) also suppressed responding dose-dependently, and was equipotent to systemically-administered quaternary naltrexone (Fig. 3). Although the largest ICV dose of tertiary naltrexone that has been evaluated, 560.0 µg (1.22 mg/kg), suppressed responding partially in all animals, the mean response rate for the five trial session was greater than 50% of the vehicle control rate. Higher doses could not be tested because doses of tertiary naltrexone greater than 100.0 µg also induced pronounced, although comparatively short-lived, shaking and vomiting.

The failure of tertiary naltrexone to suppress responding completely appeared to be related to the time course of its effects. Figure 4 shows the time course for a single ICV injection of 320.0 µg tertiary naltrexone or 56.0 µg quaternary naltrexone. While these doses produced a similar degree of rate suppression during the first trial (20–40% of control rates), the effects of tertiary naltrexone dissipated very rapidly such that responding had recovered completely by the fifth trial. Quaternary naltrexone, however, continued to suppress rates for the duration of the 1/4 hr test session. Larger doses of quaternary naltrexone (i.e., 100.0 µg) which suppressed responding completely on the day of administration, also suppressed rates 24 hr later to 41.5±13.4% of the control rate. There were no apparent rate effects 24 hr after ICV or IM tertiary naltrexone, or 24 hr after IM quaternary naltrexone.

Antagonism of Morphine’s Rate-Suppressing Effects

The potency of tertiary naltrexone in antagonizing the rate-suppressing effects of 10.0 mg/kg morphine IM was approximately the same by the two routes of administration (Fig. 5). The smallest doses of tertiary naltrexone that effectively antagonized morphine were 0.1 mg/kg IM and 0.389 mg/kg (180.0 µg) ICV. A dose of 1.0 mg/kg IM antagonized completely the suppressing effects of 10.0 mg/kg morphine, whereas doses greater than 180.0 µg tertiary naltrexone ICV did not produce additional antagonism.

Up to a dose of 32.0 mg/kg IM or 0.069 mg/kg ICV (32.0 µg), quaternary naltrexone failed to antagonize the rate-suppressing effects of 10.0 mg/kg morphine IM (Fig. 5). Larger doses were not evaluated because they suppressed response rates to less than 30% of the control rate when administered alone (Fig. 3).

**DISCUSSION**

The radiographic procedure used in this study for the periodic assessment of cannula patency allows for the incorporation of ICV drug administration in long-term behavioral experiments. Although there is a report on the use of this radiographic procedure for evaluating cannula patency in primates [10], cannula placement is most often assessed post hoc by histological techniques. These techniques, however, have the disadvantage of not permitting one to determine the cannula location until the experiment has been completed.

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**FIG. 4.** The rate-suppressing effects of a single ICV injection of 320.0 µg tertiary (diamonds) or 56.0 µg quaternary (triangles) naltrexone over a five trial (1/4 hr) test session. Abscissa: successive 15 minute trials. Other information as in Fig. 3.

**FIG. 5.** Dose-effect curves for ICV and IM tertiary and quaternary naltrexone administered prior to 10.0 mg/kg morphine (IM): closed diamonds, IM tertiary naltrexone; open diamonds, ICV tertiary naltrexone; closed triangles, IM quaternary naltrexone; open triangles, ICV quaternary naltrexone. All antagonist pretreatments were given ten minutes prior to morphine, except ICV quaternary naltrexone which was administered 60 minutes prior to morphine. The leftmost points represent the effects of 10.0 mg/kg morphine administered alone; the symbol on the left corresponds to the animals shown with the same symbol on the right. Ordinate: antagonism of morphine, expressed as a percent increase of morphine’s suppressing effects (% = effects of morphine alone (67–91% suppression of control rate); 100% = saline control rate). Abscissa: pretreatment dose of antagonist (mg/kg). Other information as in Fig. 3.
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and are, therefore, inappropriate for long-term behavioral experiments. In the absence of reliable in vivo procedures for assessing ICV cannula patency, significant time and energy could potentially be invested in animals with improper cannula placement.

It has been suggested for the pigeon that leakage of cerebrospinal fluid (CSF) when the dummy cannula is removed indicates proper placement of the cannula into the ventricle [7]. Use of this criterion alone, however, may result in a significant number of false-negative judgements of cannula patency. Leakage of CSF was observed periodically in the present study, but unambiguous positive x-rays were obtained with pigeons that never leaked CSF. The results obtained in 26 cannulated pigeons do not show a clear relationship between positive cannula placement and leakage of CSF. Although placement of the cannula tip into the lateral ventricle may not be important for all experiments (e.g., when lipophilic compounds which pass readily throughout the CNS are administered), there are undoubtedly situations in which the exact location of the cannula will influence the results that are obtained (e.g., hydrophilic, highly charged molecules which will, presumably, not diffuse readily throughout the brain). The results obtained centrally or peripherally in rats [2, 3, 18] and monkeys (Gmerek, personal communication). Based upon potency relationships for morphine given by different routes of administration, it has been argued that the rate-suppressing effects of morphine in the rat are predominantly due to its inability to pass from the CNS to the periphery. It is believed that the rate-suppressing effects of opioid antagonists in non-dependent animals are not due to actions on opioid receptors, although relatively little is known regarding the mechanism of action of antagonists in non-dependent animals. The procedure described herein may be particularly useful for comparing the mechanism(s) of action for the direct (i.e., rate-suppressing) effects of opioid antagonists in the CNS and periphery.

Potency differences of 2-300 fold have been demonstrated for various effects produced by morphine when it is administered centrally or peripherally in rats [2, 3, 18] and monkeys (Gmerek, personal communication). Based upon potency relationships for morphine given by different routes of administration, it has been argued that the rate-suppressing effects of morphine in the rat are predominantly due to its inability to pass from the CNS to the periphery. It is believed that the rate-suppressing effects of opioid antagonists in non-dependent animals are not due to actions on opioid receptors, although relatively little is known regarding the mechanism of action of antagonists in non-dependent animals. The procedure described herein may be particularly useful for comparing the mechanism(s) of action for the direct (i.e., rate-suppressing) effects of opioid antagonists in the CNS and periphery.

The results obtained in the present study were in agreement with previous reports [9,18] which indicated that lipophilic opioid antagonists (i.e., naloxone and naltrexone) are approximately equipotent when administered ICV or systemically because they diffuse easily across the BBB. Naltrexone had a similar antagonist potency whether injected centrally or peripherally, and was little if any more potent in suppressing food-maintained responding when administered ICV. Furthermore, the rapid offset of naltrexone's rate-suppressing effects when administered ICV indicates that it diffuses quickly out of the pigeon brain. This result contrasts with the long duration of rate suppression produced by large doses of quaternary naltrexone, presumably due to its inability to pass from the CNS to the periphery. It is believed that the rate-suppressing effects of opioid antagonists in non-dependent animals are not due to actions on opioid receptors, although relatively little is known regarding the mechanism of action of antagonists in non-dependent animals. The procedure described herein may be particularly useful for comparing the mechanism(s) of action for the direct (i.e., rate-suppressing) effects of opioid antagonists in the CNS and periphery.

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