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Cocaine Analgesia: An In Vivo Structure-Activity Study

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SCHUELKE, G. S., L. C. TERRY, R. H. POWERS, J. RICE AND J.A. MADDEN. *Cocaine analgesia: An in vivo structure-activity study.* PHARMACOL BIOCHEM BEHAV **53**(1) 133–140, 1996.—Hot plate testing of rats was performed to determine the optimal analgesic doses of intracerebroventricularly (ICV) administered cocaine, significant cocaine metabolites, and selected structurally similar molecules. Optimal, (subseizure) analgesic doses for cocaine and selected cocaine analogues were (in μM): cocaine, 0.37; cocaethylene, 0.09; benzoylecgonine, 0.35; norcocaine, 0.43; and ecgonine, 2.1. Ecgonine methyl ester was not analgesic at ≤ 3.7 μM. These results, in conjunction with findings on other structurally similar molecules suggest that, of the molecules tested, (a) a hydrophobic group at the C-3 attached carbon is critical for analgesia; (b) a hydrophobic C-2 ester group can enhance analgesic activity (e.g., cocaethylene); (c) the *N*-methyl position is minimally important for analgesia; and (d) isomeric configurational changes can influence analgesia.

Cocaine Benzoylecgonine Ecgonine Ecgonine methyl ester Cocaethylene Norcocaine Analgesia Nociception Tropane

HISTORICAL reports of pain-free surgery under cocaine analgesia in the early 1900s (8,11) and the use of cocaine in Brompton's cocktail (a mixture of ethanol, morphine, and cocaine) for pain relief (21) suggested that cocaine possessed central analgesic activity in addition to a local anesthetic effect. The first conclusive demonstration of a central analgesic effect of cocaine was provided by Lin et al. (18) using the hot plate thermal analgesia test in rats. Subsequently, Kiritsy-Roy, et al. (14) and Shyu et al. (31) used single unit discharge recording techniques in anesthetized rats to define a central, cocaine-sensitive, descending, dopaminergic pathway that suppressed pain-induced dorsal horn nerve activity (14,31).

Systemically administered cocaine is rapidly converted to various metabolites (10,22). Accumulating reports suggest that at least some of these metabolites can be biologically active (4,12,15,19,22,23,29). Changes in the structure of the cocaine molecule due to metabolism may render the resulting compound either more or less biologically active. The purpose of the present study was to determine the relative thermal analgesic effectiveness of cocaine, its major metabolites, and 17 tropane-ring-based analogues administered intracerebroventricularly (ICV) to rats. The compounds were administered by the ICV route to circumvent the probable inability of some of the test compounds to cross the blood–brain barrier.

METHODS

Animals and Hot Plate Test

Male Sprague–Dawley rats (Sasco, Madison, WI), weighing 150 to 300 g, were maintained in facilities certified by the American Association for the Accreditation of Laboratory Animal Care and used in accordance with standard Institutional Review procedure. Animals were maintained on a 12 h light-dark cycle with food and water ad lib. Anesthesia was induced with ketamine (Parke Davis, 87 mg/kg, IP) and xylazine (Rompun, Mobay Corp, 13 mg/kg, IP). A 25 gauge stainless steel guide cannula was implanted over the lateral cerebral ventricle (coordinates measured from Bregma: 0.8 mm posterior, 1.2 mm lateral, and 3.4 mm beneath the skull surface). Cannulas were secured to the skull with stainless steel screws (Small Parts, Miami, FL) and dental acrylic (Hygenic Corp., Akron, OH).

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Three to five days after cannula implantation (weight gain resumed) rats were injected ICV during the light portion of the 12 h cycle with either saline or one of the test compounds. Each rat was used only once. Injections were given in 10 μl total volume over 1 min via an infusion pump (Harvard Apparatus, South Natick, MA) through a 31 gauge stylet that extended 1 mm below the guide cannula.

Analgesia was assessed immediately after ICV drug injection and subsequently at 5 min intervals using an adaptation of the hot plate method of O'Callaghan and Holtzman (24). Rats were confined to a 52.5°C copper surface inside an open-ended Plexiglas cylinder (29.3 cm diameter by 27.3 cm high). Latency was recorded as the time (in seconds) from placement of the rat on the hot plate until pain sensation was indicated by either a hind paw lick or an escape jump out of the cylinder. Totally analgesic rats were removed from the hot plate at a maximum cut-off time of 45 s to minimize tissue damage.

Three base line times (latencies) were determined for each test rat immediately prior to ICV drug administration. Latencies were determined immediately after ICV injections (zero time) and at 5 min intervals for up to 120 min. Percent maximum analgesia was calculated for each postinjection time according to the following formula (18):

$$\text{Percent Analgesia} = \frac{(\text{post-ICV drug treatment response latency} - \text{average base line latency})\ 100}{(45 - \text{average base line latency})}$$

All data from any rats that demonstrated seizure activity or other behavioral changes that obfuscated pain detection were deleted from the experiment. Drug doses at which >5% of rats had to be deleted were considered to be high and a lower drug dose was used as the optimal analgesic dose. Conversly, an empirically determined drug dose that gave 80% or greater analgesia was considered sufficiently close to optimal as not to require testing of higher doses. After testing was completed, 5 μl fast green dye was injected to facilitate ventricular cannula placement as confirmed by gross necroscopy. Treated rats, selected randomly, were maintained an additional 48 h to screen for any delayed neurological effects (ataxia, sterotypy, and increased exploratory activity) or death related to the various drug treatments. None of these effects were noted during the 48 h observation period.

Each test group had a minimum of six animals. Results are expressed as mean ± SEM. Significant differences between groups of animals receiving the test compounds and control animals injected with saline only, were determined using two-way ANOVA. Differences between means were determined by Dunnett's test. The correlation between brain cocaine concentration and analgesia was determined with Pearson's correlation coefficient.

Quantitative Drug Determinations

Quantative data on either the pharmacological distribution and/or metabolic conversion of cocaine following ICV injection were not available. It was therefore necessary to confirm that cocaine itself and not a cocaine metabolite or degredation product was responsible for analgesia observed after cocaine injection. To document the presence of cocaine, six groups of rats (three to five rats per group) were injected ICV with 0.6 μM of cocaine. Rats were decapitated immediately after injection, and at 5, 10, 15, 30, 60, and 90 min postinjection. Trunk blood was collected in tubes containing NaF. Coagulation of the trunk blood was prevented by adding ethylenediaminetetraacetic acid (EDTA)/aprotinin. The plasma was separated by centrifugation at 3°C and then stored at −70°C. The brains were removed, rapidly frozen and stored at −70°C until analyzed.

Tissue drug analysis was accomplished by using urine and plasma methods adapted from Refs. 2, 3, and 26. Briefly, a 1 : 10 (w/v) homogenate was prepared in 1.0 M phosphate buffer, pH 7.0. Deuterated internal standard (D3- benzoylecgonine and D3-cocaine) was added to a 5 ml aliquot of the homogenate (30 μl/5 ml; 100 ng/μl each compound), which was then transferred to a 50 ml centrifuge tube with 10 ml distilled water and 4 g NaCl. The mixture was extracted with 10 ml chloroform/isopropanol (9 : 1, v : v) by vortexing for 30 s. Any emulsion formed was broken up by centrifugation at 1500 rpm for 5 min. The clear organic extract at the bottom of the tube was carefully removed with a Pasteur pipette and passed through 5 g anhydrous sodium sulfate into a 15 ml conical glass centrifuge tube. The mixture was extracted twice more in the same manner with 5 ml chloroform/isopropanol, and the organic extracts combined. The extract was evaporated to near dryness (a small amount of oil remained) under a stream of nitrogen in an analytical evaporator at 30°C, and the residue was derivatized with 100 μl pentafluoropropionic anhydride and 50 μl pentafluoropropnaol (70°C, 15 min) in 50 μl ethyl acetate for gas chromatography-mass spectrometry (GC-MS) analysis. Cocaine, benzoylecgonine, ecgonine methyl ester, and ecgonine in the sample extract were quantitated using a Hewlett-Packard 5890.5970 Gas Chromatograph/Mass Selective Detector by internal standard method. The chromatographic column was a 20 mm × 0.18 mm DB-5 capillary (J&W 121-5023). Cocaine (m/z 303, 182) and ecgonine methyl ester (m/z 345, 182) were quantitated using D-3 cocaine (m/z 306, 185) as the internal standard. Similarly, benzoylecgonine (m/z 421, 300) and ecgonine (m/z 463, 300) were quantitated using D-3 benzoylecgonine (m/z 424, 303) as the internal standard. All target compounds were completely resolved in our chromatographic system. Calibrators (5, 10, 50, and 100 ng/g) of each compound were prepared using control material and analyzed with each sample batch.

Plasma samples were handled similarly, except that a 2.0 ml plasma aliquot was mixed with 2 ml phosphate buffer to generate a solution analogous to the 5 ml tissue homogenate aliquot specified above. Target analytes and their corresponding ions were as detailed above. Calibrators (2, 5, 10, and 20 ng/ml) of each compound were prepared using control material and analyzed with each sample batch.

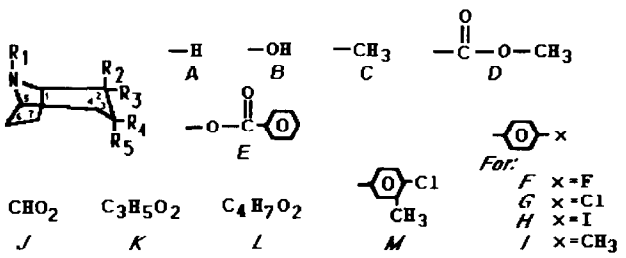
Test Compounds

Compounds were selected for testing in the analgesia assay because they were either natural metabolites of cocaine or contained modifications of the basic cocaine structure at sites that initial testing experiments suggested might be critical for activity. The compounds used are presented by structure in Table 1 and by name below. The compounds are: *1* (−) cocaine; *2* (−)-ecgonine methyl ester (EME); *3* 3β-(4-chlorophenyl) tropan-2β-carboxylic acid methyl ester tartrate; *4* 3β-(4-methyl-phenyl) tropan-2β-carboxylic acid methyl ester tartrate; *5* 3β-(3-methyl-4-chlorophenyl)-2β-carboxylic acid methyl ester hydrochloride; *6* 3β-(4′-iodo-phenyl) tropan-2β-carboxylic acid methyl ester tartrate; *7* (−)-3β-(4-fluorophenyl) tropan-2β-carboxylic acid methyl ester tartrate; *8* (+) and *9* (−)-pseudoecgonine methyl ester; *10* (−)-benzoylecgonine (BE); *11* (−)-cocaethylene (CE); *12* (+)-pseudococaine; *13* tropacocaine; *14* (−)-*N*-norcocaine (NC); *15* (−)-

TABLE 1

TROPANE RING-BASED ANALOGS

Analgesic Compounds



No.	R_1	R_2	R_3	R_4	R_5	Analgesic Dose
1	C	D	A	E	A	0.37 μM
	C3 Analogues					
2	C	D	A	B	A	>3.70 μM
3	C	D	A	G	A	0.37 μM
4	C	D	A	I	A	0.37 μM
5	C	D	A	M	A	0.37 μM
6	C	D	A	H	A	0.60 μM
7	C	D	A	F	A	0.74 μM
8	C	A	D+	B	A	2.51 μM
9	C	A	D−	B	A	4.38 μM
	C2 Analogues					
10	C	J	A	E	A	0.39 μM
11	C	K	A	E	A	0.09 μM
12	C	A	D	E	A	0.37 μM
13	C	A	A	E	A	0.74 μM
	N-Demethylation					
14	A	D	A	E	A	0.43 μM
15	CC	D	A	E	A	0.046 μM
	Multiple Position Analogues					
16	C	J	A	B	A	2.10 μM
17	C	L	A	H	A	2.22 μM
18	C	A	A	A	B	3.54 μM
19	C	A	A	A	A	3.99 μM
20	C	A	A	=O		7.18 μM
21			4-phenylpiperidine			6.20 μM

Numbers on left correspond to compound numbering in Methods. (+,−) = isomeric forms of the same molecule; *CC* = two methyl groups at the R1 position; -O designates a double bonded oxygen at the C-3 position; and the 4-phenylpiperidine structure for comparison to the core tropane ring structure above.

cocaine methiodide; *16* (−)-ecgonine (EC); *17* 3β-(4-iodophenyl) tropan-2β-carboxylic acid isopropyl ester hydrochloride; *18* tropine; *19* tropane; *20* tropinone; and *21* 4-phenylpiperidine.

Compounds 1, 2, and 18-21 were obtained from Sigma-Aldrich; the rest from NIDA. All test compounds were dissolved in sterile 0.85% saline and either used immediately or kept in the dark at 4°C for a maximum of 4 h prior to use.

RESULTS

Latencies

Mean baseline (preinjection) latency used to calculate percent analgesia for the saline control group was 11.4 ± 1.42 s (n = 22). Rats injected with all of the other test compounds exhibited similar preinjection latencies.

Cocaine and Saline Control Analgesia

Figure 1 shows the percent analgesia produced in rats injected with 0.37 μM cocaine compared to rats injected with 0.85% saline only. Rats injected with cocaine showed an almost instantaneous analgesia of 84.5% ± 12.9 that declined to 0% within 15 min. The saline control group showed an analgesic response of almost 40% at Time zero that declined to 0% within 5 min. This saline analgesia response was attributed to injection-associated stress in animals not acclimated to handling. It should be noted (Fig. 1) that with repeated testing, average analgesia responses after Time zero were negative in the saline control group. Similarly, the average analgesia responses of the cocaine group declined to zero or slightly below by 15 min (Fig. 1). Significant differences between saline and control groups were present at 0, 5, and 10 min [ANOVA; $F(3,38) = 8.0$, $p < 0.001$ and Dunnett's test]. Whether these negative responses represented a trend toward hypergesia, a rapid learning of the escape response, or random experimental variability is subject to conjecture; however, both control and test groups were subjected to the same experimental conditions.

Figure 2 demonstrates that the loss of analgesia was temporally associated with a rapid loss of ICV injected cocaine from the brain. In this experiment, a supraoptimal amount of cocaine (0.6 μM) was injected to ensure sufficient substrate for readily detectable amounts of cocaine degredation products that might be formed subsequent to ICV injection. No detectable brain levels of any other metabolite were found. These results are consistent with analgesia caused by the unaltered cocaine molecule.

The rapid cocaine loss from the brain was associated with a concomitant rise and fall of plasma cocaine and the transient appearance of low levels of plasma BE (<50 ng/ml; Fig. 3). Plasma cocaine rapidly rose to a maximum of 500 ng/ml at post-ICV injection and declined to less than 50 ng/ml by 60 min. Over the same time period a transient BE peak of slightly less than 50 ng/ml was reached at 30 min. Peripheral analgesia by the systemic levels of cocaine or metabolites attained after ICV cocaine injections was unlikely, as control intraperitoneal injections of cocaine and BE comparable to either these ng/ml plasma levels or the ICV ug amounts failed to produce analgesia (data not shown). This finding is consistent with a previous report that analgesic parenteral doses of cocaine (25 mg/kg) do not effect the conduction of impulses into the cutaneous terminals of unmyelinated nociceptive afferents (25). It is thus concluded that the analgesia associated with the ICV injection of cocaine is directly attributable to a central action of the parent cocaine molecule and not to metabolites.

Analgesia with ICV Administered Cocaine Metabolites

Results of experiments with the cocaine metabolites are presented in Fig. 4. Rats injected with BE, EC, and NC exhibited protracted analgesia relative to EME at all times between 5 and 40 min postinjection ($p < 0.05$; Dunnett's test). Removal of the *N*-methyl group to yield NC did not change the time to analgesia onset or greatly increase the dose required (0.43 μM) to attain essentially complete analgesia, but did result in an extended duration of analgesia. In contrast, BE, which has an unesterified carboxylic acid functional group at C-2 instead of the methyl ester of the cocaine molecule, had a slower onset time to maximal analgesia and the analgesic effect was protracted compared to a similar cocaine dose of 0.39 μM. A large increase in the dose required to produce optimal

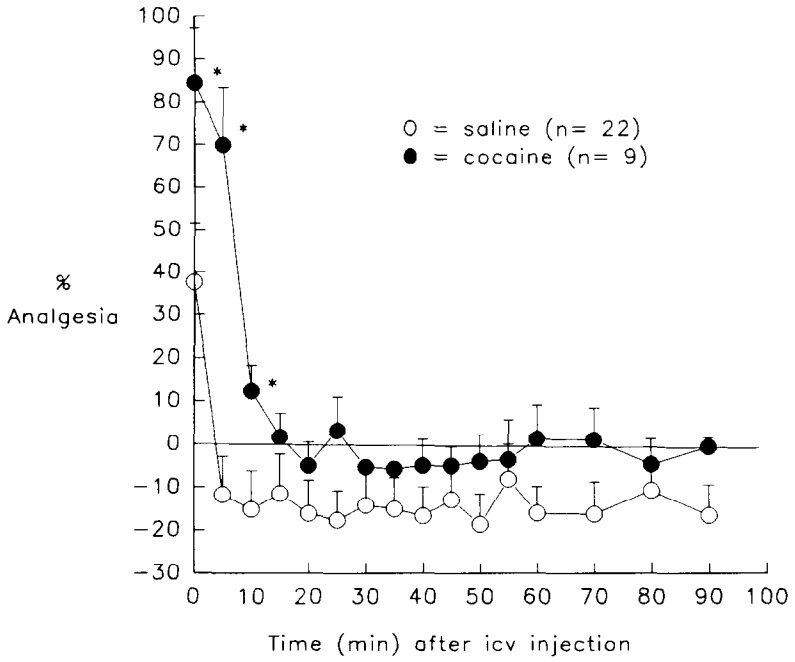


FIG. 1. Graph of percent analgesia evaluated by hot plate testing in rats injected ICV with cocaine (●) or saline (○). Values are mean ± SEM; n = 9 and 22 rats at each time point, respectively; $*p < 0.001$ compared to saline-injected rats.

analgesia was noted when the C-3 benzyl ester was absent (EC; 2.1 μM optimal analgesic dose). No analgesia was observed with EME administered at a dose 10 times (3.7 μM) the optimal dose of cocaine. These results with the natural cocaine metabolites indicate an important role for a sizable hydrophobic group at the C-3 position in generation of analgesia.

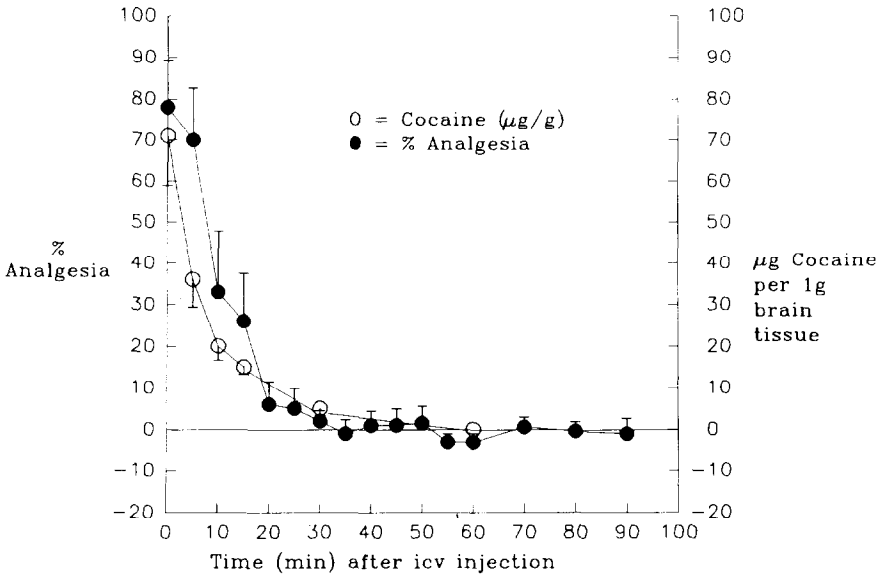


FIG. 2. Graph of percent analgesia (●) and brain cocaine concentration (○; μg/g) in rats injected ICV with cocaine. Values shown for cocaine content in brain are mean ± SEM; n = 4 brains at each time point. Values for percent analgesia are means (n = 12 rats at each time point). $p < 0.001$ by Pearson's coefficient of correlation; r = 0.93.

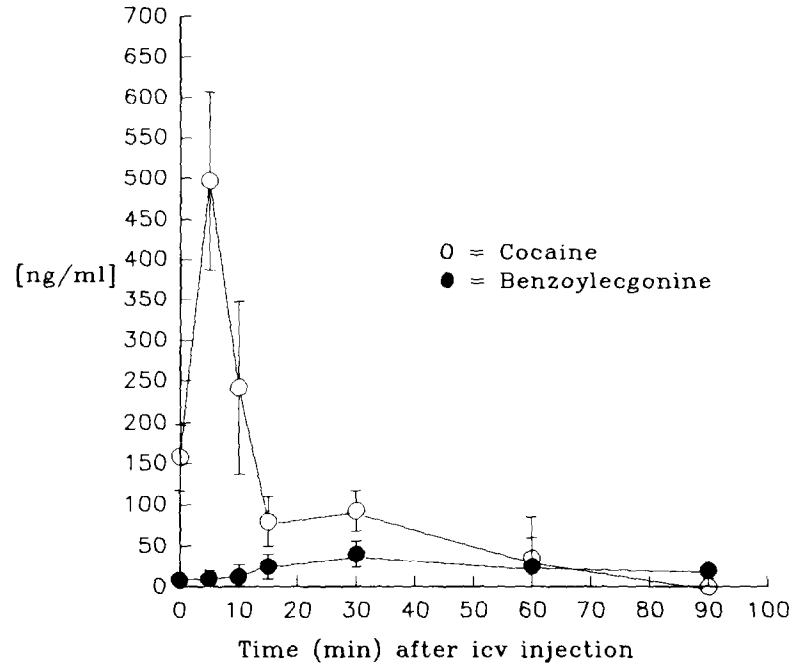


FIG. 3. Plasma concentrations of cocaine (○) and BE (●; ng/ml) after a single ICV dose of cocaine. Values are mean ± SEM.

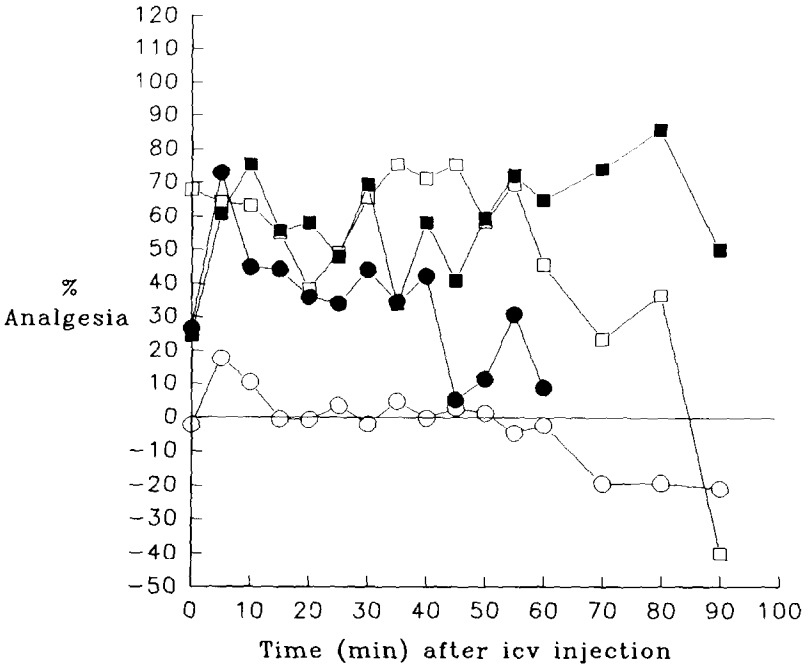


FIG. 4. Graph of percent analgesia evaluated by hot plate testing in rats injected ICV with the cocaine metabolites: BE (□; n = 8 rats at each time point), EC (●; n = 6), EME (○; n = 7), and NC (□; n = 7). Values shown are means; standard error bars have been omitted for clarity of presentation. BE, EC, and NC significantly different from EME between 5 and 40 min (Dunnett's test).

Analgesia with ICV Administered Synthetic Cocaine Analogues

Synthetic cocaine analgues with selective molecular modifications at the *N*-methyl, C-2, and C-3 positions were tested to further substantiate the initial findings with metabolites having structural variations at each of these sites. The observations with the naturally occurring cocaine metabolites were generally substantiated by the additional molecules tested, as shown in Table 1. The C-3 series of analogues support an important role of a sizable hyrophobic group at this position in determining analgesic efficacy, because only those molecules with such structures at the R4 position showed effective analgesia at doses comparable to cocaine (< 1 μM). This series of molecules also demonstrated that the ester linkage between the C-3 carbon and the ring structure is not essential for effective analgesia and that substitutions at the 3 and 4 positions of the benzene ring are not incompatable with analgesic efficacy. However, some contribution to analgesia by portions of the molecule other than just the C-3 attached benzene ring is indicated by the fact that 6.2 μM dose of 4-phenlypiperidine was needed to produce analgesia.

Quite variable results were found for molecules with changes in composition of the C-2 constituent. BE, the major cocaine metabolite with a carboxylic acid at C-2, had an analgesic efficacy equal to that of cocaine, which has a methyl ester at the C-2 carboxylic acid group. More potent however, was cocaethylene at 0.09 μM. Cocaethylene is the C-2 ethyl ester analogue of cocaine that appears to be formed when ethanol is present (9,20). In contrast, the entire elimination of the C-2 substituent (tropacocaine) resulted in an approximate 2-fold decrease in the analgesic potential compared to cocaine (0.74 μM vs. 0.37 μM). Likewise, the required analgesic dose (2.22 μM) of Compound *17*, the isopropyl analog of Compound *6*, was almost 4-fold greater than that of *6* (0.6 μM). This might indicate that there is an upper limit to the size of the C-2 ester for producing effective analgesia.

N-demethylation, which results in NC (also a major cocaine metabolite), was only slightly less potent than cocaine (0.43 μM). The only other molecule modified at the *N*-methyl position that we evaluated was cocaine methiodide *(15)*. The optimal analgesic dose was approximately 10-fold less than that of NC (i.e., 0.046 μM). That the tropane ring itself does not substantially contribute to analgesia is indicated by the 3.99 μM dose of tropane that was required for optimal analgesia. The series tropine, tropinone, and 4-phenylpiperidine further supports this concept.

Configurational changes of a molecule may or may not influence analgesia. For example, the two negative isomers, (−)-ecgonine methyl ester and (−)-pseudoecgonine ecgonine methyl ester had analgesic doses of 4.23 μM and 4.38 μM, whereas the positive isomer, (+)-pseudoecgonine methyl ester, required 2.51 μM to produce analgesia. Cocaine *(1)* and pseudococaine *(12)* differ in the orientation of the two substituent groups attached to the C-2 carbon, but are equally analgesic.

DISCUSSION

A major finding of this study was that ICV-administered cocaine was able to produce a central analgesia due to the cocaine molecule itself and not to a metabolite (Figs. 1-3). Documenting that cocaine was itself analgesic did not preclude a similar activity by other metabolites. Direct ICV injection of major cocaine metabolites showed that BE and NC also had analgesic potential at doses similar to that of cocaine (Fig. 4). These metabolites represent molecular structural changes at the C-2 and bridge nitrogen positions of the tropane ring, respectively. Molecular alterations at the C-2 position appear to potentiate analgesia. In this study, hydrolysis of the methyl ester of cocaine at C-2 to yield BE *(10)* or decarboxylation to tropacocaine *(13)*, or inversion of configuration at the C-2 position *(12)* resulted in molecules with analgesic properties essentially similar to those of cocaine.

Two other cocaine metabolites, EC and EME, had almost no analgesic activity because the doses required were approximately 7- to 10-fold greater than cocaine. Both of these compounds lack the attached benzoyl ester at the C-3 position, suggesting that a large hydrophobic group at position C-3 contributes significantly to the analgesic efficacy of the tropane analogues. Results presented in Table 1 for compounds modified at the C-3 position further support the importance of the hydrophobic group because no compound lacking it showed effective analgesic doses of less than 1 μM. Compounds 3 through 7 in Table 1 show that substitutions at the 4 position of the benzene ring are compatible with analgesic efficacy. Additionally, the ester linkage at C-3 is not essential for analgesia.

Effects of changes to the bridge nitrogen substituent position are less certain as NC was essentially as effective as cocaine in causing analgesia. However, cocaine methiodide *(15)* demonstrated comparable analgesic activity at a micromolar dose that was 10-fold lower than NC *(14)*. Possibly, the quaternary amine form remains longer in the brain (6), thereby making the molecule a more active analgesic agent. Benzoylecgonine and NC were two other compounds that produced protracted analgesia (Fig. 4). Quantative measurements of the concentrations of these molecules at sequential times after their injection were not performed. Therefore, we cannot at present differentiate between comparative altered binding efficiency to a presumed receptor molecule(s) or differences in the brain pharmacological disposition of the injected compounds.

The finding that several analogues approximate cocaine in analgesic efficacy raises two questions: (a) Might metabolites contribute to analgesia when cocaine is administered by a systemic route (intraperitoneal, intravenous, or subcutaneous)?; and (b) What are the mechanisms responsible for cocaine metabolite mediated analgesia?

The first question is addressed by our quantitative GC-MS studies of whole brain cocaine concentrations after ICV injection. Brain cocaine levels of around 30 μg/g of tissue were associated with analgesia (Fig. 2). Possible accumulations of minimally analgesic doses of polar metabolites such as BE, and lipid soluble metabolites such as NC in Sprague-Dawley rats injected systemically with cocaine is suggested by the reported finding of 10 μg NC/g brain tissue following 5 mg/kg cocaine IP (10). We found that an IP cocaine dose of $\geq$25 mg/kg were analgesic (data not shown). These doses could produce total brain NC amounts that could approach the optimal ICV-administered analgesic dose of 125 μg (0.43 μM) of NC. Thus it is likely that brain accumulations of NC, BE (though not yet experimentally demonstrated), and perhaps other metabolites, could contribute to the apparent analgesia when cocaine is administered systemically.

The mechanism of the metabolite/analogue analgesic activity in whole animals is not known. Inhibition of any single monamine uptake system does not appear to be responsible for analgesic activity because the relative in vivo analgesic capacities of the test compounds do not show obvious concordance with published in vitro binding affinities to the dopa-

mine, serotonin, or norepinephrine transporters. For example, cocaethylene was analgesic at a lower μM dose than cocaine, but has been reported to be equally effective at dopamine transporter binding (9). Similarly, (+)-pseudococaine produced analgesia at a μM dose equal to cocaine but is 40- to 180-fold less effective at binding to the DA transporter in vitro, 15 to 30 times less effective at the serotonin transporter, and 40 times less effective at the NE transporter (27,28). The optimal micromolar analgesic doses of cocaine and BE (0.37 μM vs. 0.39 μM) are even more striking because BE would be expected to be about 600, 1200, and 1100 times less active than cocaine if only binding affinities to DA, NE, or 5-HT receptors were involved (28). Part of the lack of concordance between the in vitro and in vivo results may be due to technical limitations of the whole animal assay. These greater than 1000-fold differences between receptor binding efficiencies and analgesia implies that the whole animal assay is more complex than merely the binding of a compound to a particular receptor. Possible explanations include activation of multiple receptors of decreasing affinity as drug concentrations were raised to obtain an optimal analgesic dose, physical factors such as ease of volume transmission, regional differences in receptors or receptor subtypes (17), interactions with the opoid system (13,30,32), or activation of an undefined receptor(s).

These observations of analgesia produced by cocaine metabolites could have implications for attempts to treat cocaine abuse and intoxication with methodologies, such as the use of antibodies (1,5) and/or enzymes (16) to accelerate cocaine clearance. Furthermore, development of effective clinical analgesics around tropane ring-type (7) structures remains a possibility.

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