Characteristics of the Analgesic Effects and Drug Interactions of Intrathecal Carbachol in Rats

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BACKGROUND: Intrathecal carbachol produces consistent analgesia in animals without appreciable adverse effects. Little is known about the ability of this drug to provide analgesia as stimulus intensity is increased. Likewise, there are few data regarding interactions between carbachol and other intrathecal analgesics.

METHODS: Using two different noxious radiant heat intensities, one applied to each hind limb, analgesic effects of 1, 3, 10, and 30 μg intrathecal carbachol on paw withdrawal latencies were measured. Similar testing was done for intrathecal morphine and clonidine. ED₅₀ fractions (½₅₀, ⅛₅₀, ¼₅₀, ⅛₅₀) of drug combinations of carbachol-morphine and carbachol-clonidine were administered, responses to the low intensity stimulus were recorded, and the ED₅₀ of each combination was established and isobolographic analysis of the drug interactions was carried out.

RESULTS: The 30-μg dose of carbachol was associated with transient agitation, salivation, and hind limb weakness. No other adverse effects were noted. The ED₅₀ (95% confidence interval) of intrathecal carbachol was 2.54 μg (1.34–4.69) for low intensity stimulation and 12.64 μg (4.18–38.25) for high intensity. There was no significant difference between high- and low-intensity ED₅₀ values for intrathecal morphine and clonidine. The analgesic effect of the carbachol-morphine and carbachol-clonidine combinations were significantly greater than the calculated additive effect. The ED₅₀ for the carbachol-morphine combination was 12% of the expected additive value and the ED₅₀ for the carbachol-clonidine combination was 30% of the expected additive value.

CONCLUSIONS: Intrathecal carbachol produces analgesia to noxious thermal stimulation of the hind paw in rats. It is relatively less effective at providing analgesia than intrathecal morphine or clonidine when stimulus intensity is raised. Intrathecal carbachol is synergistic when combined with intrathecal morphine or clonidine. (Key words: Anesthetic techniques: spinal. Cholinergic analgesics, intrathecal: carbachol; clonidine; morphine.)

INTRATECHAL cholinergic agonists and acetylcholinesterase inhibitors produce analgesia in animals and in humans. The analgesic effects appear to be related to muscarinic rather than nicotinic receptor activation. Intrathecal nicotinic agonists, conversely, appear to be associated with irritability and possible hyperalgesia, as demonstrated by the appearance of gnawing, vocalization, and hyperactivity after intrathecal nicotinic agonist administration. In addition, Nagiub and Yaksh observed irritability, vocalization, and trunci rigidity in animals given intrathecal neostigmine after atropine pretreatment.

Intrathecally administered cholinesterase inhibitors are synergistic with α₂-adrenergic agonists, and provide at least additive analgesia in conjunction with spinal opioids. At doses that are analgesic in human volunteers, they are associated with nausea, vomiting, and lower extremity weakness. In animals, and at large doses in humans, spinally administered neostigmine produces hypertension. The combination of very small doses of intrathecal cholinesterase inhibitors with opioids or α₂-adrenergic agents may, however, result in improved analgesia without these adverse side effects.

Several animal studies have documented the analgesic effect of carbachol (carbamyl choline) while intrathecally administered cholinesterase inhibitors were observed to produce abnormal posturing (exaggerated thoracic kyphosis and generalized irritability). Our preliminary studies of the analgesic effect of carbachol did not demonstrate these abnormalities. Therefore, we sought to determine whether carbachol, a potentially better-tolerated cholinergic agonist than neostigmine, would produce synergistic interactions with morphine and clonidine, further reducing the possibility of adverse effects.

Analgesic efficacy is often expressed as intrinsic activity, which refers to the percent receptor occupation...
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required to provide a given analgesic effect. Drugs with high intrinsic activity, such as sufentanil, are able to
produce analgesia by activation of a relatively small percent of the available opiate receptors, whereas
morphine requires a higher percent of receptor occupation. A measure of the intrinsic activity of an
analgesic drug is its ability to produce effective analgesia as the intensity of the noxious stimulus is in-
creased. Saeki and Yaksh found that there is a relatively small increase in the ED₅₀ of intrathecal sufentanil
(a drug with high intrinsic activity) to tail immersion testing as the bath temperature is increased
from 52 to 60°C, while there is a sharp increase in the ED₅₀ of intrathecal morphine (a drug with a relatively
low intrinsic activity) as the temperature is increased. Little is known about the intrinsic activity of cholin-
ergic agonists. Abram and Winne showed that intrathecal neostigmine failed to produce effective anal-
gesia on the tail immersion test at doses that produced complete analgesia to hotplate testing. The mean
control response latency to tail immersion using those paradigms was 2 s, while the mean control response
latency in the hotplate test was 10 s. This suggests that the tail immersion test produced more rapid skin heating
and that perhaps intrathecal neostigmine has a relatively low intrinsic activity. We sought to characterize
the intrinsic activity of intrathecal muscarinic agonists by assessing the dose-response characteristics of intrathecal carbachol using two different intensities of noxious thermal stimulation.

Materials and Methods

The following investigations were carried out under a protocol approved by the Animal Care Committee of the Zablocki VA Medical Center, Milwaukee, Wisconsin.

Animal Preparation

In male Sprague-Dawley rats weighing 250–350 g lumbar intrathecal catheters were implanted via an incision in the atlanto-occipital membrane under halo-
thane anesthesia as previously described by Yaksh and Rudy. Catheters were advanced 11 cm caudally and
e xternalized through the anterior portion of the scalp. Animals showing neurologic deficits on emergence from anesthesia were killed by barbiturate overdose.

To ensure correct placement of the catheters, 20 µl 2% lidocaine was injected, followed by 10 µl 0.9% saline
to flush the catheter on the day of the operation. Only animals that developed transient bilateral motor and sensory blockade in the hind legs were included in the study. Intrathecal injection studies were carried out at least 5 days postoperatively.

Drugs and Injection

All intrathecal drug doses were dissolved in normal saline and administered via micrometer driven injection device in a volume of 10 µl, followed by 10 µl saline to flush the catheter. The following drugs were used in the study: morphine (morphine sulfate; molecular weight = 324; Mallinckrodt, St. Louis, MO), clonidine (clonidine hydrochloride; molecular weight = 230.1; Sigma, St. Louis, MO), carbachol (carbamyl choline chloride; molecular weight 187.2; Sigma).

Behavioral Testing

The general behavior of all of the rats was carefully observed and tested. The following tests of motor function and coordination were carried out: observation of gait; righting reflex; and placing/stepping reflex (the dorsum of either hind paw was drawn across the edge of a table, which normally results in the animal lifting the paw and placing it on the table surface). The presence of allodynia was examined by looking for agitation (escape, aggression, or vocalization) evoked by lightly stroking the fur.

Analgesic Testing

Response latency to noxious thermal stimulation of the hind paw was assessed using a device similar to that previously reported by Hargreaves et al. Rats were confined in individual clear plastic cages placed on an elevated 2-mm thick glass surface. A movable radiant heat source (50 W, 8 V halogen projector lamp, Usio, Tokyo, Japan) with a 4-mm aperture was situated below the glass surface. The chamber below the glass was thermostatically heated to maintain the glass temperature at 30°C. The radiant heat source was positioned to focus on that portion of the plantar surface of the hind paw in contact with the glass. Activation of the radiant heat source initiated a timer. Intensities were calibrated to produce either a 5-s latency to brisk paw withdrawal (high intensity) or a 10-s latency (low intensity) in control animals. The high-intensity stimulus was used on the right hind paw of each animal, while the low-intensity one was used on the left. For the low-intensity stimulus, if an animal failed to respond within a cutoff time of 20 s, the stimulus was
terminated and a latency of 20 s was recorded. Similarly a cutoff time of 10 s was used for the high-intensity stimulus. Baseline measurements were recorded twice at each intensity and the mean was calculated.

**Study Paradigms**

After baseline testing, animals were given intrathecal injections of study drug using the following doses: carbachol 1, 3, 10, and 30 μg; morphine 0.3, 1, 3, and 10 μg; clonidine 1, 3, 10, and 30 μg. Testing was repeated at 10, 20, and 30 min after injection. These times were chosen on the basis of preliminary studies showing near maximal analgesia at these times for all three drugs tested. Five to eight animals were tested at each drug dose.

**Isobolographic Analyses**

To characterize the interactions among the drugs tested, an equal dose ratio isobolographic analysis was carried out. After calculation of the ED₅₀ for the low intensity noxious radiant heat stimulus for each drug, ED₅₀ fractions (1/2, 1/4, 1/6, 1/10) of drug combinations of carbachol-morphine and carbachol-clonidine were administered. Responses to the low-intensity stimulus were recorded at the 10-, 20-, and 30-min time intervals, and the ED₅₀ of each combination was established. These data were then compared statistically to the values that would be expected if the interactions were strictly additive.

**Statistical Analyses**

The %MPE (% maximum possible effect) for each test of response latency was calculated as:

\[
\text{postdrug latency} - \text{mean baseline latency} \times 100
\]

Calculation of the area under the time response curve from 10 through 30 min was calculated by a trapezoidal rule, and dose-response curves were constructed from the area under the %MPE curves.

The ED₅₀ values and 95% confidence intervals for individual drugs and drug combinations were calculated using the pharmacologic software programs of Tallarida and Murray. The ratios of the ED₅₀ for high-intensity versus low-intensity stimulation were compared for each drug to provide an estimate of each drug’s intrinsic activity. In addition, the %MPE for the high- and low-intensity stimulation was compared for each dose of each drug using the paired Student’s t test.

Statistical analysis of drug interactions was conducted according to procedures of Tallarida et al. The variances for the individual drugs in each mixture were calculated from the variance of the total dose. The difference between the theoretical additive point and the experimentally derived ED₅₀ was compared using the Student’s t test. For experimental values that were lower than theoretical additive values, a P value <0.05 for the differences in both the X and Y directions was interpreted as a significant synergistic interaction.

**Results**

**Behavioral Effects**

No behavioral abnormalities were noted for any of the morphine-treated animals or for any animals receiving carbachol-morphine or carbachol-clonidine combinations. Animals receiving 30 μg intrathecal carbachol exhibited mild transient agitation for 10-12 min after injection (6 of 8 animals), mild, transient (10-15 min) hind limb paresis (3 of 8), and salivation (4 of 8). Transient conjunctival bleaching was noted just after intrathecal injection in one animal at that dose. No animals receiving smaller doses of carbachol exhibited any adverse effects. Animals that received 10 or 30 μg intrathecal clonidine exhibited brisk diuresis after injection.

**Analogic Studies**

The ED₅₀ values for both high- and low-intensity stimulation of the three drugs tested are shown in table 1. The ratio of the high- to low-intensity ED₅₀ values for carbachol (5.40) is greater than that of either morphine (2.15) or clonidine (2.12). There was no overlapping of the 95% confidence limits for the high- and low-intensity ED₅₀ values of carbachol and there were significant differences between the high- and low-intensity analogic effects for the 3, 10, and 30 μg carbachol doses (P < 0.05; figs. 2A and 2B). No differences in analogic effects were observed for the carbachol-morphine or carbachol-clonidine combinations. The ED₅₀ values for the morphine-clonidine combination were: morphine 1.13 ± 0.03 μg and clonidine 1.17 ± 0.06 μg. For the analogic effect, the experimental and control values were 1.13 ± 0.01 μg clonidine and 0.79 ± 0.03 μg clonidine. None of these differences were significant for both drugs.

**Discussion**

It is generally assumed that the effect of clonidine analogues is due to a receptor effect rather than nicotinic receptors. The fact that is the assumption is fairly clear in many studies where a nitroprusside agonist (5) is producing analogic effects seems to suggest that the analogic effect is a nitroprusside receptor-mediated event that can be reversed by a specific antagonist (6). The observed analogic effects of carbachol and clonidine in this study are consistent with the findings of other studies (7), although the analogic effects were not as marked as those observed in this study. The reason for this is not clear.
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Bach doses ($P < 0.05$; fig. 1A). There was considerable overlapping of the 95% confidence intervals for the high- and low-intensity EDSO values for both morphine and clonidine and there were no significant differences in analgesic effect between the high- and low-intensity stimuli for any dose of those drugs (figs. 1B and 1C).

Isobolographic Analyses

For both the carbachol-morphine and carbachol-clonidine combinations, the combined effect of the drugs was significantly greater than the calculated additive effect (figs. 2A and 2B). Experimental values that lie inside the line of additivity, which connects the EDSO values of the two drugs, are considered to have greater-than-additive, or synergistic effects. For the carbachol-morphine combination, the experimental EDSO values (±SEM) were: morphine $0.06 ± 0.01$ µg; carbachol $0.15 ± 0.03$ µg. The expected additive values for this combination are: morphine $0.51 ± 0.01$ µg; carbachol $1.17 ± 0.06$ µg. For the carbachol-clonidine combination, the experimental EDSO values were: clonidine $0.23 ± 0.01$ µg; carbachol $0.33 ± 0.03$ µg. The expected additive values for this combination are: clonidine $0.79 ± 0.03$ µg; carbachol $1.17 ± 0.05$ µg. The differences between additive and experimental values were significant for both drug combinations ($P < 0.05$).

Discussion

It is generally assumed that the intrathecal analgesic effect of cholinergic agonists is the result of muscarinic rather than nicotinic receptor stimulation. Support for this assumption is fairly convincing. The highly specific muscarinic agonist (+)-cis-methyldioxolane is capable of producing analgesia on both the hotplate and tail-flick tests.\textsuperscript{12} Most studies evaluating the analgesic effect of intrathecal nicotine fail to demonstrate an appreciable analgesia,\textsuperscript{1,6,9} although one study showed a fleeting ($<5$ min) analgesic effect.\textsuperscript{18} In addition, intrathecal atropine,\textsuperscript{1,2,4,6,8,10} but not mecamylamine,\textsuperscript{6} reliably reverses or prevents the analgesic effects of intrathecal cholinergic agonists.

Intrathecal carbachol produces profound analgesia to moderate levels of noxious thermal stimulation with minimal adverse effects. This is in contrast with neostigmine, which produces prolonged irritability and abnormal posturing at doses that are less profoundly analgesic.\textsuperscript{12} The reason for the lack of side effects of

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*Fig. 1. Dose-response curves for high and low intensity radiant heat stimulation. (A) Carbachol; (B) morphine; (C) clonidine. *Significant difference between high- and low-intensity values ($P < 0.05$, paired Student’s $t$ test).*
it is thought to have at least some nicotinic effect.\textsuperscript{15} It is not clear whether the side-effect profile of carbachol in humans would be different from that of neostigmine, whose principle side effect is nausea and vomiting. Neither drug is associated with vomiting in rat studies.

Carbachol is relatively ineffective at producing analgesia when stimulus intensity is increased to produce rapid skin heating. At the largest dose tested (30 \(\mu\)g), the analgesic effect on the high-intensity stimulus was only about 60\% (larger doses were not tested because of the occurrence of profound motor blockade, marked agitation, and salivation at doses higher than 30 \(\mu\)g in preliminary studies). This suggests that intrathecal cholinergic agonists may be relatively ineffective in treating severe cancer pain, or intense incident pain, such as rib fracture. Another characteristic of drugs with low intrinsic activity is a profound loss of effect with continuous or repetitive administration, \textit{i.e.}, rapid development of tolerance.\textsuperscript{20} Svensson \textit{et al.}\textsuperscript{3} demonstrated that tolerance to analgesia for both thermal and mechanical nociception developed after 5 days of daily intrathecal carbachol injections (10 \(\mu\)g). These observations suggest that cholinergic agonists may not be ideal analgesic agents for long-term use.

While the relative inability of carbachol to provide analgesia at the higher stimulus intensity suggests that it may not be an ideal analgesic, the dramatic reduction in doses associated with combining the drug with either morphine or clonidine is quite encouraging. This effect suggests that postoperative intrathecal morphine doses might be dramatically reduced by the addition of carbachol, reducing the risk of respiratory depression and possibly reducing the incidence of side effects of either drug. Likewise, combinations of carbachol and clonidine may be useful in patients who experience severe opioid side effects or who have become tolerant of opioids.

The ability of intrathecal morphine to produce analgesia at the higher stimulus intensity in this study was surprising in light of previous studies demonstrating that drug's very poor analgesic effects with high-intensity stimuli.\textsuperscript{15,21} We suggest a possible explanation for this discrepancy: The high-intensity stimulus we used may not have been intense enough to test the upper limits of morphine's efficacy (although it was obviously adequate to do so for carbachol). In the studies by Sacki and Yaksh\textsuperscript{15} and Yaksh,\textsuperscript{21} the cutoff times for the high-intensity stimuli were the same as for low intensity, while our cutoff times were adjusted to twice the baseline for each stimulus. Therefore, the high-
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intensity stimuli in their studies probably represented a much more marked increase in stimulus intensity, perhaps to the point of tissue injury.

The potential of intrathecal carbachol to produce neurologic damage in humans remains an issue. The drug has been used in many rat studies without reports of adverse effects except for transient hind limb weakness at large doses. A neurotoxicologic study carried out in rats receiving intrathecal carbachol for 14 days failed to demonstrate any histologic differences between carbachol- and saline-treated animals. However, more extensive testing in other species would be required before the drug can be tested in humans. That there are no commercially available preservative-free preparations of carbachol makes it unlikely that it will be made available for human use. However, two other muscarinic agonists, betahanechol and methacholine, are in clinical use, and may prove to have analgesic applications if safety and efficacy can be established.

The authors thank Drs. James Fujimoto, Blythe Holmes, and Jody Rudy for their assistance and advice.

References