Production of Analgesia by Cholinergic Drugs (37164)

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A central cholinergic mechanism has been implicated in the production of analgesia by narcotic agents (1, 2). Parasympathomimetic agents depress operant behavior in several species without depressing escape behavior (3, 4) and have been reported to potentiate analgesia induced by morphine and related drugs (1, 5–7). Moreover, recent studies have indicated that some cholinergic compounds may exert direct analgesic effects (2, 8–11). The present study was undertaken using the anticholinesterase agents neostigmine and physostigmine, and the direct cholinomimetic pilocarpine to determine the extent of analgesia provided by these agents alone and to determine whether or not the muscarinic blocking agents atropine and scopolamine would antagonize this effect.

Methods. The radiant heat "tail-flick" was used to determine analgesia and to quantitate tolerance to physostigmine sulfate and pilocarpine hydrochloride. The apparatus used was constructed as a metal box containing a ventilation fan and a small asbestos lined circular opening at the top. Just below this opening a nickel wire coil serving as the heat source was extended and controlled by a remote power switch operated in conjunction with a stopwatch. The mean, control reaction time of 382 male Holtzman rats (150–158 g nonfasted) used in this study was 5.62 ± 0.04 (SEM) sec. Reaction times were assessed immediately before injection of drugs and 30 min after injection of drugs. The EAD_{50} was defined as the dosage (mg/kg) of drug that increased reaction time by 50% in one-half of the animals tested; each animal served as his own control. Each animal in the tolerance study received two EAD_{90} doses (50% increase in the reaction time in 90% of the animals tested) of physostigmine on the first day. There was a 5 hr interval between the two doses. On Days 2, 3 and 4 each animal received, in two daily injections, twice the amount of drug administered on Day 1. Pilocarpine animals were treated similarly. Drugs were freshly prepared in saline (0.9% NaCl) and administered in a 2 ml/kg injection volume (sc). Atropine sulfate and scopolamine hydrochloride were tested for antagonism of analgesia induced by pilocarpine and physostigmine. The $EADB_{50}$ was defined as the dose of scopolamine or atropine that inhibited analgesia in 50% of the animals given an EAD_{99} dose of pilocarpine or physostigmine. The EAD_{50}, EAD_{90}, EAD_{99} and $EADB_{50}$ values for all drugs used in this study are expressed as the weight of the salt form of each drug. The effective dose and 95% confidence limits for each drug tested were determined by the statistical methods of Litchfield and Wilcoxon (12).

Results. Physostigmine and pilocarpine produced analgesia when administered in acute studies. The EAD_{50} for physostigmine was 0.13 mg/kg and 5.7 mg/kg for pilocarpine (Table I). Neostigmine bromide failed to produce any measurable analgesic effect with doses between 0.12 and 0.24 mg/kg. This dose range did produce serious side effects and closely approached the LD_{50} for the drug. Animals pretreated with multiple daily doses of physostigmine and pilocarpine exhibited a significant increase in the EAD_{50} (Table I). The EAD_{50} for morphine sulfate in animals tolerant to physostigmine or pilocarpine was not different from the EAD_{50} of morphine (4.0 mg/kg) in normal animals. Scopolamine and atropine inhibited analgesia produced by pilocarpine and physostigmine (Table II). The $EADB_{50}$ of atropine was 21 times greater for physostigmine than pilo-

TABLE I. Tolerance to Analgesia Induced by Physostigmine and Pilocarpine.

Pretreatment	EAD_{50} (mg/kg) sc	
	Day 0	Day 5
Physostigmine[c]	0.13[a] (0.10–0.17)[b]	0.2 (0.18–0.23)
Saline[e]	—	0.13 (0.09–0.16)
Pilocarpine[d]	5.7 (4.7–7.8)	9.4 (7.9–11.3)
Saline	—	5.6 (4.8–7.4)

[a] Each EAD_{50} value was obtained based on experiments in from 20 to 25 animals.

[b] mg/kg, sc (95% confidence limits).

[c] On Day 1 each animal received 0.36 mg/kg of drug and 0.72 mg/kg daily on Days 2–4.

[d] On Day 1 each animal received 16 mg/kg of pilocarpine and 32 mg/kg daily on Days 2–4.

[e] All control animals were injected daily with normal saline (2 ml/kg) on an injection schedule which paralleled the experimental animals.

carpine given in equianalgesic doses. The $EADB_{50}$ of scopolamine was the same for physostigmine and pilocarpine. Scopolamine was 5 and 100 times more potent than atropine in blocking analgesia induced by physostigmine and pilocarpine, respectively. No antinociceptive effect was induced by scolopamine or atropine and neither of these drugs was effective in blocking analgesia produced by the EAD_{99} dose of morphine (7.8 mg/kg).

Discussion. The results indicate that on a milligrams per kilogram basis the analgesic potency of physostigmine is 35 times that of morphine, while pilocarpine showed potency comparable to morphine. Scopolamine was more potent than atropine in blocking the analgesia produced by the cholinergic drugs. These data are in accord with previous work which showed that these two drugs exert qualitatively similar effects on the central nervous system (3, 13). The difference in potency may be due to the more efficient diffusion into the brain of scolopamine rather than to the differences between the central and peripheral effects of the two drugs (3, 14). The fact that neostigmine failed to produce an analgesic effect discounted a peripheral mechanism of action, since it is well known that quaternary nitrogen compounds enter the CNS with great difficulty.

Significant tolerance developed over a short time to the analgesia produced by both physostigmine and pilocarpine. The acute EAD_{50} of physostigmine (0.13 mg/kg) did not induce cholinergic side effects, *viz,* salivation, lacrimation, rapid respiration, diarrhea or tremors. However, these effects were observed in each animal that received an equianalgesic dose of pilocarpine (5.7 mg/kg). Tolerance developed to the side effects of pilocarpine as previously demonstrated after repeated administration of neostigmine (15).

These data are in accord with those shown by Hendershot and Forsaith (9) indicating that in mice—using the phenylbenzoquinone writhing test—a positive depression of response occurred with both physostigmine and pilocarpine. The increased reaction time to painful stimuli following the administration of drugs infers analgesia in the phenylbenzoquinone writhing test (16) mentioned above,

TABLE II. Antagonism (by Atropine and Scopolamine) of Analgesia Induced by Pilocarpine and Physostigmine.

Analgesic treatment[a]	$EADB_{50}$[b]; mg/kg sc	
	Atropine	Scopolamine
Physostigmine[c] (0.18 mg/kg)	0.21 (0.12–0.36)[e]	0.002 (0.001–0.004)
Pilocarpine[d] (8.0 mg/kg)	0.01 (0.006–0.015)	0.002 (0.001–0.004)

[a] Effective analgesic dose, sc, simultaneously with atropine or scopolamine, 30 min prior to analgesic test.

[b] Effective analgesic dose blockade in 50% of animals that received EAD_{99} of physostigmine or pilocarpine.

[c] Simultaneous injection of 2 mg/kg of 0.9% saline with physostigmine (EAD_{99}) 30 min prior to analgesic test does not affect the reaction times measured.

[d] Simultaneous injection of 2 mg/kg (0.9%) saline with pilocarpine (EAD_{99}) 30 min prior to analgesic test does not affect the reaction times measured.

[e] mg/kg (95% confidence limit), each experiment value obtained with 26–46 animals.

as well as the rat "tail-flick" assay (7), the mouse hot-plate jumping test (17) and the electroshock test (18). The mechanism of analgesia of these drugs apparently involves a central cholinergic mechanism; however, neither scopolamine nor atropine altered morphine analgesia. Therefore, it is not unreasonable to suggest that analgesia can be induced by at least two different mechanisms.

Summary. Pilocarpine and physostigmine exhibit analgesia in the rat radiant heat tail-flick assay. This analgesia may be blocked by scopolamine or atropine. The mechanism of the analgesia produced by these cholinergic agents is of a central and not peripheral origin. Animals develop tolerance to the analgesic effects of both these drugs with chronic administration. Neostigmine fails to produce measurable analgesia.

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