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Analgesia Following Microinjection of Phosphodiesterase Inhibitors at Brainstem Sites

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LEVY, R. A. AND B. D. GOLDSTEIN. *Analgesia following microinjection of phosphodiesterase inhibitors at brainstem sites.* PHARMAC. BIOCHEM. BEHAV. 15(3) 501–504, 1981.—Microinjection of RO 20-1724 (0.60 μg) at sites in the caudal reticular formation of the rat brainstem resulted in analgesia as measured in the tail flick, paw pinch and hot plate tests. Microinjection of theophylline (0.45 μg) at sites in the caudal reticular formation also produced analgesia in the tail flick and hot plate tests, but not in the paw pinch test. Since these agents inhibit phosphodiesterase and elevate cyclic nucleotides in rat brain homogenates, these data suggest that cyclic nucleotides have a functional role in brainstem structures involved in modulation of nociceptive threshold.

Analgesia Brainstem Microinjection Phosphodiesterase inhibitors Cyclic nucleotides

WE have previously shown [7,8] that the dibutyryl derivatives of cyclic 3′:5′ adenosine and guanosine cyclic monophosphates (db cAMP and db cGMP) cause analgesia when microinjected at sites in the caudal reticular formation (CRF) of the rat brainstem. This suggests that endogenous cyclic nucleotides may be involved in synapses of pathways which modulate the nociceptive threshold. It is possible, however, that this analgesic action of exogenously applied db cAMP and db cGMP reflects an alteration of transmission at synapses which do not normally utilize cyclic nucleotides. In the present experiments we have applied the phosphodiesterase inhibitors RO 20-1724 and theophylline locally at CRF sites in order to elevate levels of endogenous cyclic nucleotides. The analgesia observed following such application suggests that endogenous cyclic nucleotides are involved in the modulation of nociceptive threshold by activity in structures located in the CRF.

METHOD

Cannula Implantation

Stainless steel guide sheaths (22 ga) were implanted in Sprague-Dawley or Holtzman rats (either sex, 225–410 g) under pentobarbital (45 mg/kg) anesthesia. The sheath was stereotaxically positioned about 3.0 mm above the target area in the caudal brainstem reticular formation (CRF). The CRF coordinates were P 3.0 to 3.3, H 1.0, L 0.0 or 1.0; incisor bar −2.5 mm. The guide sheath was attached to the skull with jeweler's screws and dental acrylic and fitted with a 28 gauge stylet to prevent occlusion. Animals were housed individually after surgery and allowed at least one week for recovery.

Microinjection Procedure

Drugs were microinjected at the target sites through a 28 gauge stainless steel injection cannula inserted through and extending about 3.0 mm beyond the guide sheath. Drugs were delivered in a fixed volume (0.5 μl) of vehicle over a period of about one minute. Drug solutions were injected with a 10 μl syringe, attached to the cannula with PE 20 polyethylene tubing. Flow was monitored by observing the movement of an air bubble over a calibrated length of the tubing. The cannula remained in place for 1 min after injection to minimize back flow of drug up the sheath. Each animal received one microinjection.

RO 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) was dissolved in a solution of 3.8% ethanol in 0.9% NaCl (pH 6.7). Theophylline was dissolved in 0.9% NaCl (pH 6.5). Solutions of RO 20-1724 and theophylline were prepared immediately before use.

Analgesia Testing

Changes in nociception were assessed using the tail flick

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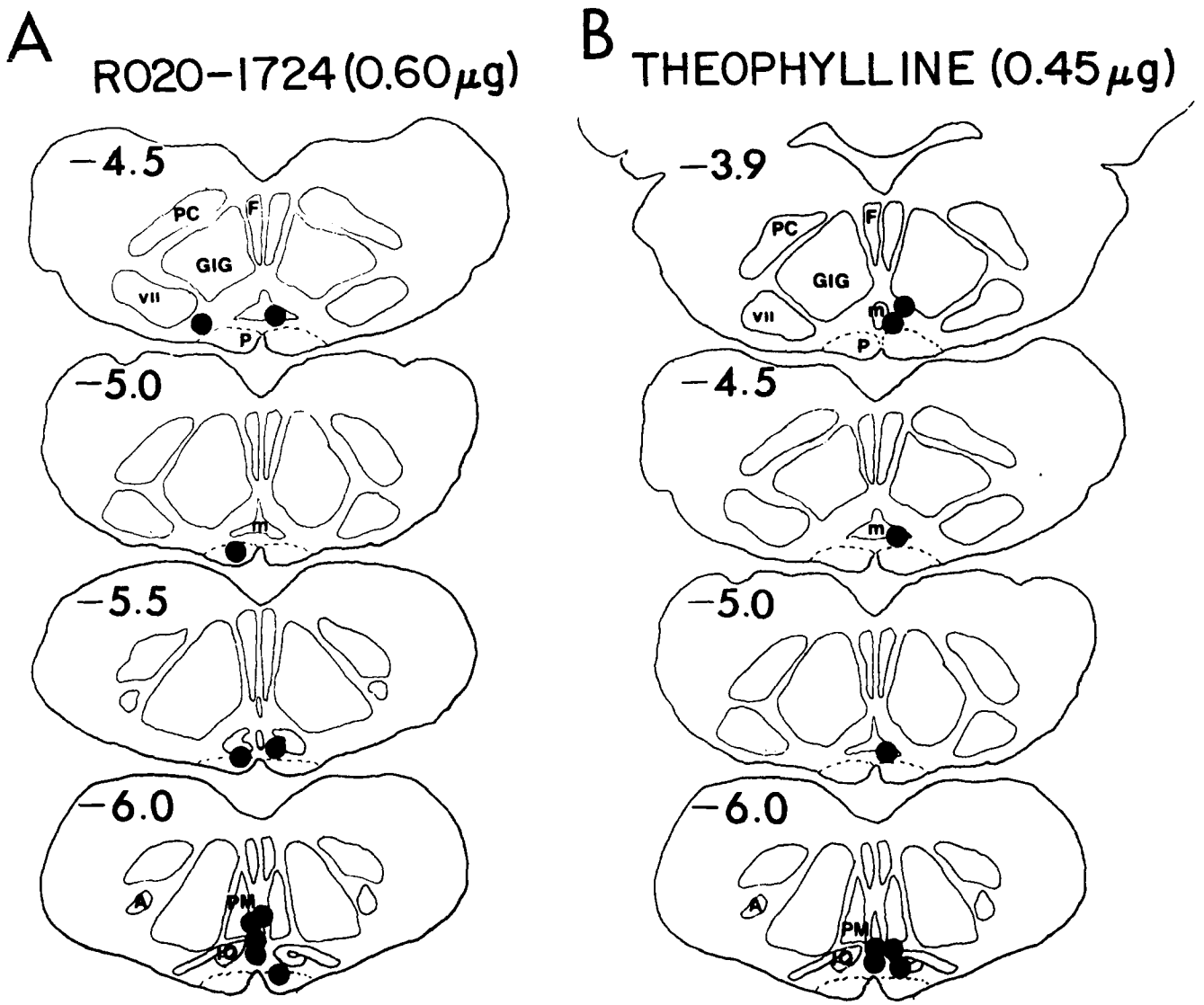

FIG. 1. Sites in the caudal reticular formation at which RO 20-1724 (A) and theophylline (B) were microinjected. GIG, nucleus reticularis gigantocellularis; PC, nucleus reticularis parvocellularis; m, nucleus raphe magnus; F, median longitudinal fasciculus; VII, nucleus of the seventh nerve; P, pyramidal tract; IO, inferior olive; A, nucleus ambiguous. The negative numbers in each coronal section indicate the distance (mm) caudal to the interaural line.

(TF), paw pinch (PIN), and hot plate (HP) tests prior to and at fixed intervals of 10, 15, 30, 45, 60 and 90 minutes following microinjection of RO 20-1724 and ethanol and 5, 12.5, 30, 45, 60 and 90 minutes following microinjection of theophylline at CRF sites. The effect of the 3.8% ethanol solution on nociception following injection at CRF sites was assessed in control experiments performed in an additional group of animals. Analgesia was first assessed on the TF test, in which the tail was placed over a resistance heating element of nichrome wire. The time which elapsed between onset of the heat and the reflex removal (flick) of the tail was defined as the tail flick latency. The average of three successive determinations was recorded.

Nociception was next tested using the PIN test, by placing the hindpaw between the jaws of a pincer; one jaw was flat and the other cone-shaped. The force exerted by the pincer was increased at a constant rate of 64 g/sec. The time at which the rat removed the paw, or struggled to do so, was recorded. The average of two successive determinations, one from each hindpaw, was taken as the latency for paw withdrawal. The animal was held during this test but not restrained.

Animals were then placed on a 55°C hot plate and the time required to lick a hindpaw, jump, or vocalize was recorded as the hot plate latency. One determination was recorded. Animals which failed to respond by 14 sec on the TF test, by 15.6 sec on the PIN test, or by 40 sec on the HP test, were removed, to minimize tissue damage, and assigned the maximum value for that test.

Location of Injection Sites

The location of the injection site in each rat was verified

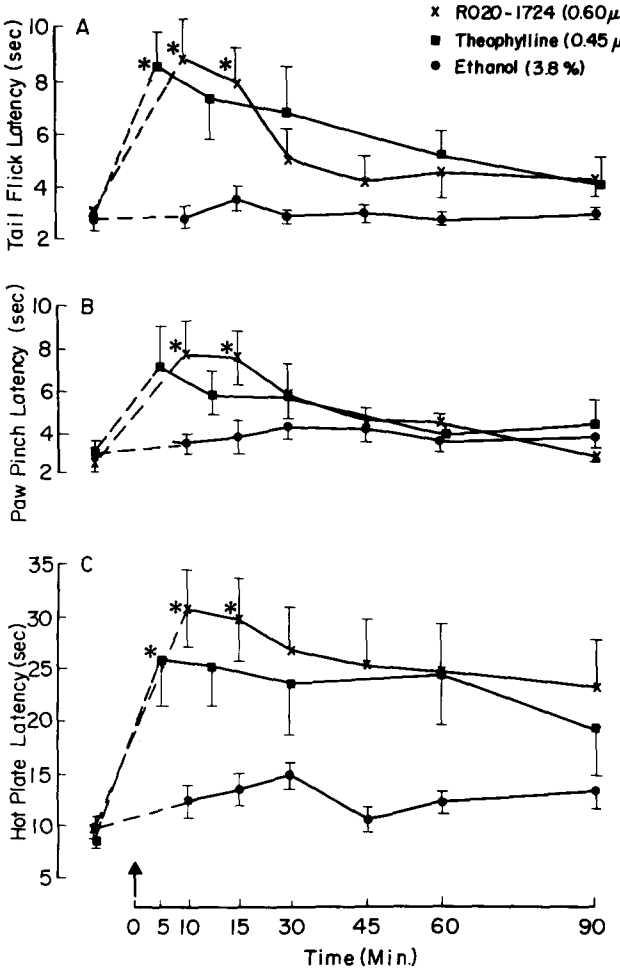

FIG. 2. Tail flick (A), paw pinch (B), and hot plate (C) latencies following microinjection (arrow) of RO 20-1724 (n=10) and theophylline (n=8) at the caudal reticular formation sites shown in Fig. 1. Latencies following microinjection of ethanol vehicle for RO 20-1724 at an additional 11 sites (not illustrated in Fig. 1) are also shown. Each point represents mean±SEM. *$p<0.05$, compared with pre-drug control value (Newman-Keuls test).

from 10 μm frozen sections stained with cresyl violet and the position indicated on coronal sections drawn from the atlas of Palkovits and Jacobowitz [11].

Statistical Analysis

Alterations in sensitivity to noxious stimuli produced by RO 20-1724, theophylline, and ethanol were evaluated using one-way analysis of variance [6]. Comparisons of pre- vs post-drug mean response latencies were made using the Newman-Keuls test for multiple post-hoc comparisons at individual time points [6].

RESULTS

Preliminary experiments were conducted to identify a dose of RO 20-1724 that would alter sensitivity to noxious stimuli following microinjection at sites in the CRF. Microinjection of 0.02 μg/0.5 μl RO 20-1724 (0.15 mM), a concentration less than that which has been reported to suppress phosphodiesterase activity *in vitro* [2], caused a modest elevation in HP latency but failed to alter nociception on the TF or PIN tests. A clear analgesic effect was observed at a dose of 0.60 μg/0.5 μl (4.5 mM) and the results using this dose are reported in detail below.

RO 20-1724 (0.60 μg) was injected at a total of 10 sites in the caudal brainstem (Fig. 1A). One-way analysis of variance indicated that analgesia occurred in the TF, PIN, and HP tests ($p<0.05$ for all tests) following microinjection at these sites (Fig. 2). Maximal analgesia was seen after ten min, the earliest sampled post-injection time point. Analgesia was still evident at 20 min but not present at 45 min or at subsequent intervals. The analgesic efficacy of 3.8% ethanol in saline, the vehicle in which the RO 20-1724 solution was dissolved, was tested following microinjection at 11 sites in the CRF (not illustrated), similar to those sites shown in Fig. 1. No analgesic action was observed following microinjection of ethanol at these sites on either the TF, PIN, or HP tests ($p>0.1$ for all tests).

Animals injected with RO 20-1724 usually showed no motor disturbance, although some turning behavior was seen in 3 of the 10 animals represented in Fig. 1A. We did not judge this disturbance to be of sufficient magnitude to disrupt performance on any of the analgesiometric tests. Ataxia was seen in an additional three animals which also showed analgesia following microinjection of RO 20-1724. These animals were deleted from the study, since in these cases assessment of nociception may have been obscured by a motor deficit. Ethanol caused ataxia in three animals, and caused analgesia in two of these three. These three animals were also not represented in the data of Figs. 1 and 2.

Microinjection of 5 mM theophylline (0.45 μg/0.5 μl) at 8 CRF sites (Fig. 1B) caused analgesia in the TF and HP tests ($p<0.05$ in both tests) (Fig. 2). Analgesia was present only at 5 min, the first sampled post-injection time point. Previous results [5] indicated that microinjection of 0.5 μl saline control solutions at CRF sites did not cause elevations in the nociceptive threshold in the TF or HP tests. Analgesia was not observed in the PIN test following microinjection of 0.45 μg theophylline at CRF sites (Fig. 2). Microinjection of 0.9 μg (n=7) or 1.8 μg (n=4) theophylline at similar sites in the CRF also did not cause analgesia in the PIN test (not illustrated). Microinjection of 0.9 μg (n=7) or 1.8 μg (n=4) theophylline at CRF sites also did not cause analgesia in the TF test (not illustrated), although analgesia was observed on this test following the lower dose of 0.45 μg, as mentioned above. No motor effects were observed following microinjection of theophylline in any of the tested doses.

DISCUSSION

These results indicate that analgesia is produced following microinjection of both RO 20-1724 and theophylline at sites in the caudal reticular formation. Both of these agents have been shown to inhibit phosphodiesterase and to elevate cyclic nucleotides [14]. Theophylline inhibits both cyclic AMP and cyclic GMP phosphodiesterase, whereas RO 20-1724 selectively inhibits cyclic AMP phosphodiesterase [3]. Phosphodiesterase inhibition was reported in these studies to occur at concentrations less than those injected locally in the present experiments. However, the actual concentration at the affected brainstem structures following local microinjection is unknown since it depends on various factors govern-

ing the diffusion of these materials to the structure from a point source of application.

The results suggest that cyclic nucleotides may be involved in synaptic transmission in caudal brainstem structures involved in the modulation of nociception. Such structures include the nucleus gigantocellularis and nucleus raphe magnus [4, 10, 12]; many of the injection sites of Fig. 1 are located either within or in close proximity to these structures. The more consistent demonstration of analgesia with RO 20-1724 compared to theophylline may reflect the greater potency of RO 20-1724 as a phosphodiesterase inhibitor [14]. It is possible, however, that the analgesic action of theophylline and/or RO 20-1724 observed in the present experiments may only partially involve inhibition of phosphodiesterase. RO 20-1724, for example, has been reported to also inhibit adenosine uptake [9], and adenosine analogs cause analgesia following intracerebroventricular administration [15]. However, the cyclic nucleotide system may be indirectly involved here since adenosine has been shown to elevate intracellular levels of cAMP [13]. Theophylline, on the other hand, has been shown to antagonize the action of adenosine [13] in smaller concentrations than those required for phosphodiesterase inhibition [1]. Theophylline, therefore, would not be expected to cause analgesia but on the contrary to lower the nociceptive threshold, if its principle action in the present experiments was to antagonize the action of endogenous adenosine. It is likely therefore, that the analgesia presently observed following microinjection of theophylline at CRF sites reflects its action as a phosphodiesterase inhibitor. It is of interest in this regard that 0.45 μg theophylline caused analgesia in the tail flick test but higher doses of theophylline failed to cause analgesia in this test. These observations suggest the possibility of multiple dose-related actions when theophylline is applied locally in brainstem structures which regulate the nociceptive threshold.

The present results, showing that the phosphodiesterase inhibitors theophylline and RO 20-1724 cause analgesia when injected at CRF sites, along with our observation that dibutyryl cyclic AMP and cyclic GMP also cause analgesia when applied at CRF sites [7,8], suggest that endogenous adenosine and guanosine cyclic nucleotides mediate synaptic transmission in brainstem structures involved in modulation of the nociceptive threshold.

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