Pergamon

Pharmacology Biochemistry and Behavior, Vol. 48, No. 1, pp. 37–42, 1994

0091-3057/94 $6.00 + .00

Nicotine-Induced Antinociception in Mice: Role of G-Proteins and Adenylate Cyclase

M. I. DAMAJ, S. P. WELCH AND B. R. MARTIN[1]

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0613

Received 20 May 1993

DAMAJ, M. I., S. P. WELCH AND B. R. MARTIN. *Nicotine-induced antinociception in mice: Role of G-proteins and adenylate cyclase.* PHARMACOL BIOCHEM BEHAV **48**(1) 37–42, 1994.—The effects of pertussis toxin, forskolin, and cAMP analogues on the antinociceptive action of nicotine were examined to investigate the possible involvement of adenylate cyclase and G-proteins in nicotine's antinociceptive effect. Intrathecal injection of pertussis toxin (0.25 and 0.50 μg) in mice inhibited nicotine-induced antinociception in the tail-flick test. The effect of the toxin was dose and time dependent. Forskolin, a potent adenylate cyclase activator, and 8-(-4-chlorophenylthio) adenosine-3′ : 5′ monophosphate, cyclic (8-CPT-cAMP), a cAMP analogue, inhibited the antinociceptive effects of nicotine in a dose-dependent manner. EGTA reversal of 8-CPT-cAMP's inhibitory effects suggests that calcium may to be involved. These data implicate the possible involvement of a G-protein and a second messenger system (activation of a cAMP-dependent protein kinase and increase in cyclic AMP levels) in nicotine-induced analgesia in mice.

Nicotine Cyclic AMP Antinociception G-Proteins Mice Forskolin Calcium

THE nicotinic cholinergic receptor of the mammalian neuromuscular junction is probably the most thoroughly characterized of what are referred to as ligand-gated ion channels. By comparison, not much is known about related nicotinic receptor subtypes present in the central nervous system (CNS). An important aspect of the functionality of the various nicotinic acetylcholine receptor (nAChR) subtypes is the signal transduction mechanism involved in the activation of neuronal cholinergic receptors (nAChR). Unfortunately, our understanding of the signaling processes associated with the nicotine receptor has been confined largely to the peripheral nervous system. Stimulation of nAChRs in chromaffin cells and muscle induce membrane depolarization which results in an influx of calcium and an increase in intracellular calcium concentrations (18,25,26). Nicotine has been shown also to elevate cAMP levels in rat and bovine adrenal medullary cells and in PC-12 cells (2,3,13). It is also important to point out that phosphorylation of nAChR in muscle by agents that enhance cAMP levels, such as forskolin or cAMP analogs, involves cAMP-dependent protein kinase and results in an acceleration of the rate of desensitization of the receptor (16).

In the CNS, certain neuronal nicotinic receptors have a substantial permeability to calcium ions (10,23,33) and their function is regulated by calcium ions acting on the outside of the cell (24). Furthermore, our laboratory has shown that L-type calcium channel blockers are able to block nicotine-induced behavioral effects in mice and that calcium channel activators enhance nicotine's effects (8). However, there is little direct evidence describing the involvement of adenylate cyclase and cAMP in the transduction mechanism of neuronal nAChR.

In the present study, we have examined the involvement of G-proteins and c-AMP by determining the effect of pertussis toxin, an irreversible inactivator of a number of G-proteins including G_i (linked to adenylate cyclase) and G_o (linked to ion channels) (14,30) and forskolin, which enhances cAMP levels by activating adenylate cyclase; on the antinociception produced by systemic administration of nicotine. The antinociceptive effect of nicotine is thought to be mediated by a central mechanism, because this effect is antagonized by mecamylamine, but not hexamethonium (21,27,32).

METHOD

Animals

Male ICR mice (20–25 g) obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. They were housed in groups of six and had free access to food and water.

[1] To whom requests for reprints should be addressed.

Drugs

(−)-Nicotine was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) and converted to the ditartrate salt as described previously (1). [^{3}H]-(−)-Nicotine (80 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Dibutyryl-cAMP (db-cAMP), EGTA, pertussis toxin and 1,9 dideoxyforskolin were purchased from Sigma (St. Louis, MO). Forskolin, 7β-deacetyl-7β-(g-*N*-methylpiperazino)-butyryl was purchased from Calbiochem (La Jolla, CA). 8-(-4-Chlorophenylthio)adenosine-3′ : 5′ monophosphate, cyclic (8-CPT-cAMP) was purchased from Boehringer Mannheim (Indianapolis, IN). (±)-BAYK 8644 was obtained from Research Biochemical Incorporated (Natick, MA). All the drugs were dissolved in physiological saline (0.9% sodium chloride) except for (±)-BAYK 8644 (dissolved in DMSO) and pertussis toxin which was dissolved in 50% glycerol containing 50 mM sodium phosphate and 0.5 M NaCl. All the drugs were injected intrathecally (IT) except for (−)-nicotine, which was injected SC. All doses are expressed as the free base of the drug.

Antinociceptive Assay and Experimental Design

Nicotine-induced antinociception in mice was measured by the tail-flick procedure (9,11). A control response (2–4 s) was determined for each animal before treatment. A maximum latency of 10 s was imposed to prevent damage to the tail. Mice were retested 5 min after SC administration of either saline or nicotine. The antinociceptive response was calculated as percent MPE, where percent MPE = [{(test-control)/(10-control)} × 100]. In the pertussis toxin experiments, mice were randomly assigned to a vehicle (50% glycerol containing 50 mM sodium phosphate, 0.5 M NaCl, pH = 7.2), or pertussis toxin group (0.25–0.5 μg/mouse). Following a single pretreatment with an IT injection of either vehicle or toxin, the antinociceptive effect of nicotine was determined 2–7 days later. db-cAMP, 1,9 dideoxyforskolin, forskolin and 8-CPT-cAMP were injected 5 min before nicotine administration. To investigate a possible involvement of calcium in cAMP effects on nicotine, mice were pretreated with EGTA, a calcium chelator, and with (±)-BAYK 8644, a calcium channel activator. The first group of mice received EGTA intrathecally, followed 20 min later by IT 8-CPT-cAMP, then 5 min later a SC challenge dose of nicotine. The second group of mice received an IT injection of (±)-BAYK 8644 followed 5 min later by an IT injection of 8-CPT-cAMP and then by a SC dose of nicotine.

Intrathecal Injections

Intrathecal injections were performed free hand between the L5 and L6 lumbar space in unanesthetized male mice according to the method of Hylden and Wilcox (17). The injection was performed using a 30-gauge needle attached to a glass microsyringe. The injection volume in all cases was 5 μl. The accurate placement of the needle was evidenced by a quick flick of the mouse's tail. In protocols where two sequential injections were required in an animal, the flicking motion of the tail could be elicited with each subsequent injection. Thus, the accurate placement of all injections could be assured by watching the tail motion of the mouse.

[^{3}H](−)-Nicotine Binding In Vitro

[^{3}H](−)-Nicotine binding assays were performed in vitro according to the method of Scimeca and Martin (29) with minor modifications. Tissue homogenate was prepared from whole mouse brain (minus cerebellum) in 10 volumes of ice-cold 0.05 M Na-K phosphate buffer (pH 7.4) and centrifuged (17,500 × *g* 4°C) for 30 min. The pellet was then resuspended in 20 volumes of ice cold glass-distilled water and allowed to remain on ice for 60 min before being centrifuged as before. The resulting pellet was then resuspended to a final tissue concentration of 10 mg/ml of buffer. Membranes from whole brain (0.2 ml of final suspension) were incubated at 4°C for 2 h with phosphate buffer and [^{3}H]-nicotine in a total volume of 1 ml. Nonspecific binding was determined in the presence of 100 μM unlabeled (−)-nicotine. The incubation was terminated by rapid filtration through a Whatman GF/C glass fiber filter (presoaked overnight in 0.1% poly L-lysine to reduce radioligand binding to the filters). Filters were washed twice with 3 ml of the buffer, and radioactivity on the filters was measured using a liquid scintillation spectrometer. Displacement of [^{3}H]-nicotine binding was determined in the presence of increasing concentrations of db-cAMP, 1,9 dideoxyforskolin, forskolin, and 8-CPT-cAMP.

Statistical Analysis

Data were analyzed statistically by an analysis of variance followed by the Dunnett *t*-test. The null hypothesis was rejected at the 0.05 level. ED_{50} and AD_{50} values with 95% CL and ED_{84} values were determined by the method of Litchfield and Wilcoxon (20).

RESULTS

Effect of Pertussis Toxin

The effect of pretreatment of mice with IT pertussis toxin (0.25 and 0.5 μg/mouse) 2–7 days prior to evaluating the antinociceptive effect of 1.5 mg/kg SC of nicotine (ED_{84}) is illustrated in Fig. 1. Treatment of the mice with 0.25 and 0.5 μg/mouse of pertussis toxin (PTX) IT did not modify the basal tail-flick latencies but led to a loss of antinociceptive potency of nicotine. This reduction was evident the second day after PTX pretreatment for the dose of 0.5 μg and was still fully present 7 days later. The toxin effect was dose dependent, 0.5 μg being more effective than 0.25 μg to reduce nicotine-

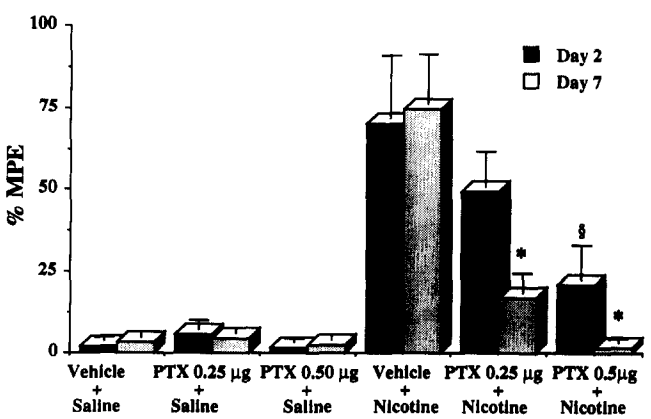

FIG. 1. Effect of pretreatment (day 2 and 7) with pertussis toxin (0.25 and 0.50 μg/mouse, IT) on the antinociceptive action of nicotine (1.5 mg/kg, SC) in the tail-flick test. PTX = pertussis toxin. Results are expressed as mean (± SE) of percent MPE. §Significantly different from the nicotine group day 2 at $p < 0.05$. *Significantly different from the nicotine group day 7 at $p < 0.05$.

induced antinociception. The loss in the antinociceptive effect of nicotine was not significant on day 2 using a dose of 0.25 μg/mouse of PTX (70 ± 19% MPE and 50 ± 11% MPE in nicotine- and pertussis toxin-pretreated mice, respectively), but became significant on day 7 of pretreatment. The effect of nicotine was completely abolished on day 7 after 0.5 μg of PTX.

Body weights of mice used in both control and nicotine experimental groups were recorded. There was no significant reduction in body weight in mice pretreated with 0.5 μg PTX (88 ± 3% of control day 2, 90 ± 7% of control day 7).

Effect of Forskolin and Its Analog

Pretreatment (IT) with forskolin reduced the effect of nicotine (1.5 mg/kg SC) in the tail-flick test in a dose-dependent manner (Fig. 2). The AD_{50} of forskolin was 0.04 μg [$(2.7 \times 10^{-2}$–$5.4 \times 10^{-2})$] or 98 pmol/animal. Forskolin alone did not significantly alter the tail-flick latencies produced following administration of any of the doses tested in this experiment. Pretreatment with IT 1,9 dideoxyforskolin (10 μg/animal), an analog of forskolin that does not activate adenylate cyclase, significantly attenuated the antinociceptive effect of 1.5 mg/kg of nicotine (Fig. 3, 75 ± 16% MPE and 31 ± 15% MPE in nicotine- and 1,9 dideoxyforskolin-pretreated mice, respectively, $p < 0.55$). 1,9 Dideoxyforskolin alone did not produce any significant effect on the tail-flick latencies.

Effect of cAMP Analogs

An IT pretreatment with db-cAMP, an analog of cAMP, did not significantly reduce the effect of nicotine in the tail-flick test. Increasing the dose of db-cAMP to 50 μg did not reveal any significant reduction in nicotine's antinociceptive effect (Fig. 4). However, IT pretreatment with 8-CPT-cAMP, another analog of cAMP, did reduce significantly nicotine's effect in mice. The dose-response curve of nicotine-induced antinociception was shifted (Fig. 5) to the right by 1 μg of 8-CPT-cAMP, and the ED_{50} of nicotine was increased from 1.05 mg/kg (0.90–1.68) to 2.30 mg/kg (1.70–3.05). Pretreatment with 5 μg of 8-CPT-cAMP shifted nicotine's dose-response further to the right, but it was not possible to obtain complete dose-response curves at that dose due to the toxicity of higher doses of nicotine.

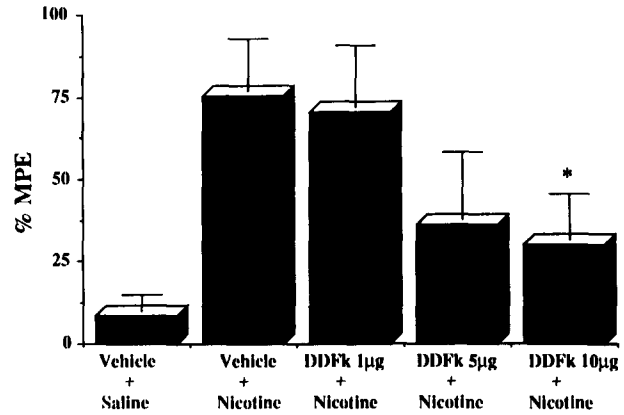

FIG. 3. Effects of pretreatment with 1,9 dideoxyforskolin (1, 5, and 10 μg given intrathecally) on the antinociception induced by nicotine (1.5 mg/kg, SC) in mice as measured by the tail-flick test. DDFK = 1,9 dideoxyforskolin. Results are expressed as mean (±SE) percent MPE. *$p < 0.05$ from the nicotine group.

Involvement of Calcium in the 8-CPT-cAMP Inhibitory Effect on Nicotine-Induced Antinociception

EGTA, the calcium chelator, given IT at the dose of 0.3 μg was able to attenuate the inhibitory effect of 1 μg of 8-CPT-cAMP on nicotine's antinociceptive action (1.5 mg/kg, SC) (Fig. 6A). On the other hand, 0.1 μg IT of (±)-BAYK 8644, a calcium channel activator, significantly enhanced the action of a subactive dose of 8-CPT-cAMP (0.025 μg/IT) on nicotine's effect (Fig. 6B). By themselves, EGTA and (±)-BAYK 8644 did not produce antinociception at the indicated doses and times, nor they did significantly alter the antinociceptive effects of nicotine or 8-CPT-cAMP.

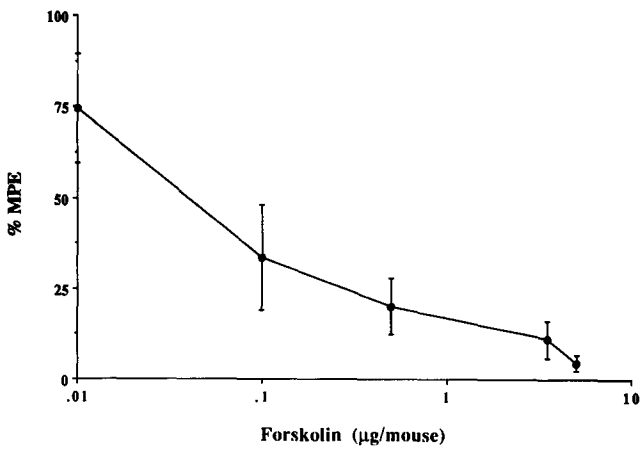

FIG. 2. Effect of IT administration of forskolin upon nicotine-induced antinociception in mice. Nicotine (1.5 mg/kg) was administered SC and the mice were tested 5 min later. Each point represents the mean ± SE of six to eight mice. *$p < 0.05$ from vehicle (open circle).

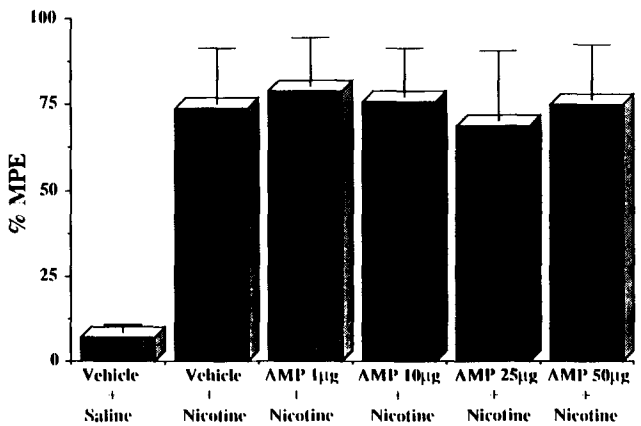

FIG. 4. Effects of pretreatment with dibutyryl-cAMP (1, 10, 25, and 50 μg given intrathecally) on the antinociception induced by nicotine (1.5 mg/kg, SC) in mice as measured by the tail-flick test. AMP = dibutyryl-cAMP. Results are expressed as mean (±SE) percent MPE.

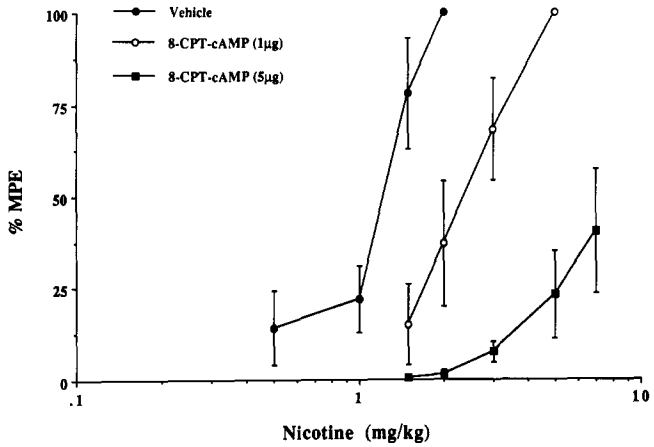

FIG. 5. Effects of 8-(-4-chlorophenylthio)adenosine-3′ : 5′ monophosphate, cyclic (8-CPT-cAMP) upon the dose-response curve of nicotine-induced antinociception. The dose-response for nicotine was established in the presence of 8-CPT-cAMP (1 and 5 μg given intrathecally). Results are expressed as mean (± SE) percent MPE.

Binding Studies

The Scatchard analysis of [^{3}H]-nicotine binding provided a K_D of 4.7 ± 1.2 nM and B_{max} of 253 ± 56 fmol/mg protein. db-cAMP, 1,9 dideoxyforskolin, forskolin, and 8-CPT-cAMP at 1 and 10 μM concentrations did not displace [^{3}H]-nicotine binding.

DISCUSSION

In the present study, the consistent inhibition of nicotine's effect by IT pertussis toxin suggests an involvement of a neural substrate at the spinal level in the regulation of the antinociceptive activity mediated by the nicotinic receptor. Pertussis toxin irreversibly inactivates a number of G-proteins, including G_i (linked to adenylate cyclase) and G_o (linked to ion channels) (14,30). Previous studies have suggested that nicotine-induced dopamine release in PC-12 cells (7) and in bovine adrenal cells (4) is regulated via pertussis toxin-sensitive G-proteins. Using chick myotubes, Eusebi et al. (12) observed that application of GTP-γS, a nonhydrolyzable analogue of GTP that irreversibly activates all G-proteins, causes a decrease in the frequency of opening of nAChR channels as well as a decrease in their conductance.

Based on these data and on the in vivo model used, it is difficult to ascertain whether PTX is modulating nicotinic function by a direct involvement with a G-protein (G_o) or an indirect action through a second messenger system activated by a G-protein (G_i linkage to adenylate cyclase). PTX could be working at nicotinic or nonnicotinic receptor-linked G-proteins or at any G-protein associated with other modulators of the neurons or even other neurons involved in the antinociceptive effects of nicotine.

Our findings with forskolin and the cAMP analog 8 CPT-cAMP indicate that an activation of a cAMP-dependent protein kinase and increase in cyclic AMP levels have an inhibitory effect on nicotine-induced analgesia in mice. The possibility of a direct interaction with the nicotine binding site is unlikely, due to the inability of forskolin and 8-CPT-cAMP to displace [^{3}H]-nicotine binding. It is also well established that agents that enhance cAMP levels, such as forskolin or

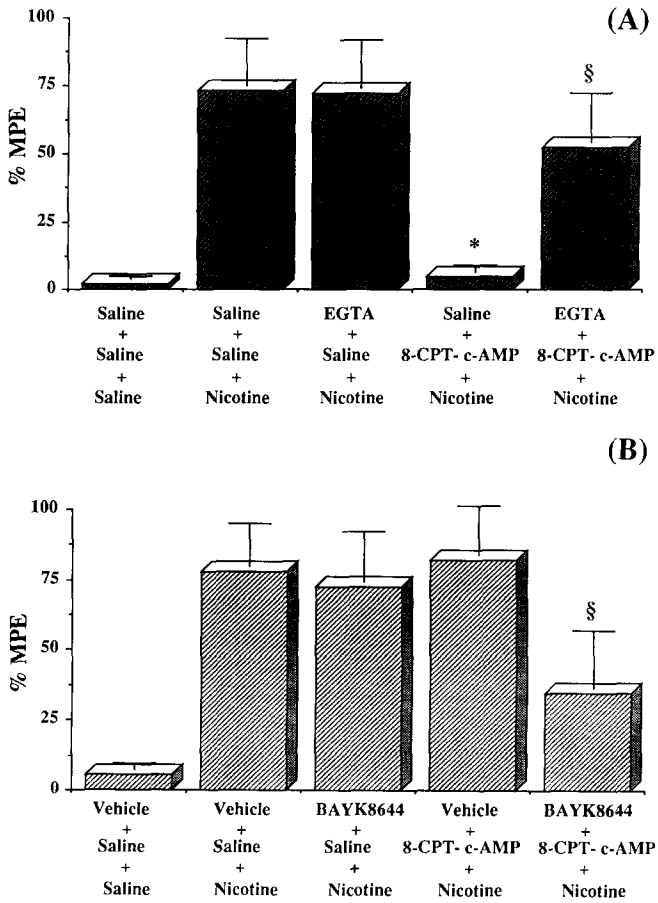

FIG. 6. Effects of IT pretreatment with either EGTA (panel A) or (±)-BAYK 8644 (panel B) on the inhibitory effect of 8-CPT-cAMP on nicotine-induced antinociception (1.5 mg/kg, SC) in mice as measured by the tail-flick test. EGTA (0.3 μg IT) and (±)-BAYK 8644 (0.1 μg IT), were followed 20 and 5 min later, respectively, by IT 8-CPT-cAMP (1 μg for the EGTA group and 0.025 μg for the BAYK 8644 group), then 5 min later a SC challenge dose of nicotine was administered. Results are expressed as mean (± SE) of percent MPE. §Significantly different from the 8-CPT-cAMP-nicotine group at $p < 0.05$. *Significantly different from the nicotine group at $p < 0.05$.

cAMP analogs, stimulate the phosphorylation of nAChR in rat myotubes, bovine adrenal chromaffin cells, and chick ciliary ganglion neurons (5,16,34). Phosphorylation of nAChR could involve cAMP-dependent protein kinase and result in an acceleration of the rate of desensitization of nAChR (16). However, regulation of the rate of desensitization has only been reported for the peripheral nicotinic receptor (16).

Interpretation of forskolin's effect on nicotine antinociception is complicated by the fact that this drug, in addition to activating adenylate cyclase at low concentrations, has direct local anesthetic-like effects on a variety of ion channels, including the nAChR and potassium channels, and at higher concentrations enhances nicotinic receptor desensitization (Torpedo receptors, myotubes) and inhibits glucose transport (19,35). However, the inability of 1,9 dideoxyforskolin (a naturally occurring analog of forskolin that does not activate adenyl cyclase) to inhibit nicotine's effect at relevant doses would argue for a cAMP-dependent effect of forskolin. The

significant attenuation of the antinociceptive effect of nicotine at a very high dose (250-fold higher than the AD_{50} of forskolin) of 1,9 dideoxyforskolin could be due to the nonspecific effects on K^+ (15) and calcium channels (37) or on membrane transport proteins (19). In addition, White et al. (36) recently showed that the cAMP-independent effects of forskolin on current activated by high concentrations of GABA were not mimicked by 1,9-dideoxyforskolin.

Findings with 8-CPT-cAMP should be interpreted with some caution. Recent studies have shown that the cAMP analog is a potent inhibitor of the cGMP-specific phosphodiesterase (6), and that cA kinase and cG kinase are triggered simultaneously (28). In addition, the failure of db-cAMP to alter nicotine's antinociceptive effects appears to contradict the actions of 8-CPT-cAMP. However, db-cAMP has failed to mimick cAMP in other systems known to be regulated by cAMP (22,31). Although db-cAMP readily penetrates cells, there are some concerns regarding the nonspecific effects of its hydrolysis product. One explanation could be that butyrate, the hydrolysis product of db-cAMP, interfered with the second messenger systems (38), which has been reported for gene expression of the β-adrenergic receptor, chromatin-associated protein kinase, and cytosolic pH. Failure of db-cAMP to have an effect in other systems known to be regulated by cAMP as reported could be related to interference from butyrate. Taken together, the observations with pertussis toxin and 8-CPT-cAMP suggest that some of nicotine's behavioral effects may be mediated, in part, by a signal transduction mechanism where cAMP production is involved. Additionally, the cAMP analog's action on nicotine-induced antinociception appears to be regulated by calcium fluxes because EGTA and BAYK 8644 were able to modulate this effect. Thus, an interaction between intracellular calcium and cAMP could play a role in signal transduction following activation of different neuronal nicotinic subtypes.

In conclusion, our data reinforces the possible involvement of a G-protein and a second messenger system in neuronal nAChR activation. Investigation of such mechanisms in different models, for example in vitro biochemical and electrophysical systems, would be very important.

ACKNOWLEDGEMENTS

The authors greatly appreciate the technical assistance of Gray Patrick and Bin Yang. This work was supported by National Institute on Drug Abuse grant DA-05274 and DA-06031.

REFERENCES

1. Aceto, M. D.; Martin, B. R.; Uwaydah, I. M.; May, E. L.; Harris, L. S.; Izazola-Conde, C.; Dewey, W. L.; Vincek, W. C. Optically pure (+) nicotine from (±) nicotine and biological comparison with (−) nicotine. J. Med. Chem. 22:174–177; 1979.
2. Anderson, K.; Robinson, P. J.; Marley, P. D. Cholinergic regulation of cyclic AMP levels in bovine adrenal medullary cells. Br. J. Pharmacol. 107:360–366; 1992.
3. Baizer, L.; Weiner, N. NGF treatment enhances nicotine-stimulated dopaminergic release and increases cyclic AMP levels in PE12 cell cultures. J. Neurosci. 5:1176–1179; 1985.
4. Bansal, M.; Phillips, T.; Van Heyningen, S. The inhibition by pertussis and tetanus toxins of evoked catecholamine release from intact and permeabilized bovine adrenal chromaffin cells. FEBS Lett. 165:165–168; 1990.
5. Cheek, T.; Burgoyne, R. Cyclic AMP inhibits both nicotine-induced actin disassembly and catecholamine secretion from bovine adrenal chromaffin cells. J. Biol Chem. 262:11663–11666; 1987.
6. Connolly, B.; Willits, P.; Warrington, B.; Murray, K. 8-(4-chlorophenyl) thio-cyclic AMP is a potent inhibitor of the cyclic GMP-specific phosphodiesterase (PDE VA). Biochem. Pharmacol. 44:2303–2306; 1992.
7. Courtney, N.; Howlett, A. C.; Westfall, T. Dopaminergic regulation of dopamine release from PC12 cells via a pertussis toxin-sensitive G protein. Neurosci. Lett. 122:261–264; 1991.
8. Damaj, M. I.; Martin, B. R. Calcium agonists and antagonists of the dihydropyridine type: Effect on nicotine-induced antinociception and hypomotility. Drug Alcohol Depend. 32:73–79; 1993.
9. D'Amour, F. E.; Smith, D. L. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72:74–79; 1941.
10. Decker, E. R.; Dani, J. A. Calcium permeability of the nicotinic acetylcholine receptor: The single-channel calcium influx is significant. J. Neurosci. 10:3413–3420; 1990.
11. Dewey, W. L.; Harris, L. S.; Howes, J. F.; Nuite, J. A. The effect of various neurohormonal modulations on the activity of morphine and the narcotic antagonists in tail-flick and phenylquinone test. J. Pharmacol. Exp. Ther. 175:435–442; 1970.
12. Eusebi, F.; Grassi, F.; Molinaro, M.; Zani, B. M. Acetylcholine regulation of nicotinic receptor channels through a putative G-protein in duck myotubes. J. Physiol. 393:635–645; 1987.
13. Guidotti, A.; Costa, E. A role for nicotinic receptors in the regulation of the adenylate cyclase of adrenal medulla. J. Pharmacol. Exp. Ther. 189:665–675; 1974.
14. Hidebrandt, J. D.; Sekuna, R. D.; Codina, J.; Lyengar, R.; Van Clark, C. R.; Birnbaumer, L. Stimulation and inhibition of adenyl cyclases mediated by distinct regulatory proteins. Nature 302: 706–709; 1983.
15. Hoshi, T.; Garber, S. S.; Aldrich, R. W. Effect of forskolin on voltage-gated K^+ channels is independent of adenylate cyclase activation. Science 240:1652; 1988.
16. Huganir, R.; Greengard, P. Regulation of neurotransmitter receptor desensitization by protein phosphorylation. Neuron 5:555–567; 1990.
17. Hylden, J. L.; Wilcox, G. L. Intrathecal morphine in mice : A new technique. Eur. J.Pharmacol. 67:313–316; 1980.
18. Kilpatrick, D. L.; Slepetis, R. J., Corcoran, J. J.; Kirshner, N. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. J. Neurochem. 38:427–435; 1982.
19. Laurenza, A.; Sutkowski, E.; Seamon, K. Forskolin: A specific stimulator of adenylyl cyclase or a diterpene with multiple sites of action? Trends Pharmacol. Sci. 10:442–447; 1989.
20. Litchfield, S. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
21. Martin, T. J.; Suchocki, J.; May, E. l.; Martin, B. R. Pharmacological evaluation of the antagonism of nicotine's central effects by mecamylamine and pempidine. J. Pharmacol. Exp. Ther. 254: 45–51; 1990.
22. Middleton, P.; Jaramillo, F.; Schuetze, S. Forskolin increases the rate of acetylcholine receptor desensitization at rat soleus endplates. Proc. Natl. Acad. Sci. USA 83:4967–4971; 1986.
23. Mulle, C.; Choquet, D.; Korn, H.; Changeux, J.-P. Calcium influx through nicotinic receptor in rat central neurons: Its relevance to cellular regulation. Neuron 8:135–143; 1992.
24. Mulle, C.; Clement, L.; Changeux, J.-P. Potentiation of nicotinic receptor response by external calcium in rat central neurons. Neuron 8:937–945; 1992.
25. Noronha-Blob, L.; Gover, R.; Baumgold, J. Calcium influx mediated by nicotinic receptors and voltage sensitive calcium channels in SK-N-SH human neuroblastoma cells. Biochem. Biophys. Res. Commun. 162:1230–1235; 1989.
26. O'Sullivan, A. J.; Cheek, T. R.; Moreton, R. B.; Berridge, M. J.; Burgoyne, R. D. Localization and heterogeneity of agonist-

induced changes in cytosolic calcium concentration in single bovine adrenal chromaffin cells from video imaging of fura-2. EMBO J. 8:401–411; 1989.
27. Sahley, T.; Berntson, G. Antinociceptive effects of central and systemic administrations of nicotine in the rat. Psychopharmacology (Berlin) 65:279–283; 1979.
28. Sandberg, M.; Butt, E.; Nolte, C.; Fisher, L.; Halbügge, M.; Jahnsen, T.; Genieser, H.-G.; Jastorff, B.; Walter, U. Characterization of Sp-5,6-dichloro-2-β-D-ribofuranosylbenzini-dazole-3′,5′-monophosphorothioate(Sp-5,6-DCI, CBIMPS) as a potent and specific activator of cyclic-AMP-dependent protein kinase in cell extracts and intact cells. Biochem. J. 279:521–525; 1991.
29. Scimeca, J. A.; Martin, B. R. The effect of acute Dyflos (DFP) treatment on[^{3}H]nicotine binding to mouse brain homogenate. J. Pharm. Pharmacol. 40:793–797; 1988.
30. Stryer, L.; Bourne, H. R. G proteins: A family of signal transducers. Annu. Rev. Cell. Biol. 2:391–419; 1986.
31. Treistman, S. N.; Levitan, I. B. Alterations of electrical activity in molluscan neurones by cyclic nucleotides and peptides factors. Nature 261:62–64; 1976.
32. Tripathi, H. L.; Martin, B. R.; Aceto, M. D. Nicotine-induced antinociception in ratsand mice: Correlation with nicotine brain levels. J. Pharmacol. Exp. Ther. 221:91–96; 1982.
33. Vermino, S.; Amador, M.; Luetje, C. W.; Patrick, J.; Dani, J. A. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron 8:127–134; 1992.
34. Vijayaraghavan, S.; Schmid, H.; Halvorsen, S.; Berg, D. Cyclic AMP-dependent phosphorylation of a neuronal acetylcholine receptor a-type subunit. J. Neurosci. 10:3255–3262; 1990.
35. White, M. Forskolin alters acetylcholine receptor gating by a mechanism independent of adenylate cyclase activation. Mol. Pharmacol. 34:427–430; 1988.
36. White, G.; Li, C.; Ishac, E. 1,9-Dideoxyforskolin does not mimick all cAMP and protein kinase A independent effects of forskolin on GABA activated ions currents in adult rat sensory neurons. Brain Res. 586:157–167; 1992.
37. Yanagibashi, K.; Papadopoulos, E.; Iwaki, T; Kawamura, M. Forskolin activates voltage-dependent Ca^{2+} channels in bovine but not in rat fasciculata cells. Endocrinology 124:2383–2387; 1989.
38. Yusta, B.; Ortiz-Caro, J.; Pascual, A.; Aranda, A. Comparaison of the effects of forskolin and dibutyryl cyclic AMP in neuroblastoma cells: Evidence that some of the actions of dibutyryl cyclic AMP are mediated by butyrate. J. Neurochem. 51:1808–1814; 1988.