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Pharmacological Characterization of the Novel ACh Releaser α -tropanyl 2-(4-bromophenyl)propionate (PG-9)

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Learning—Memory—Pain—PG-9.

INTRODUCTION

Ghelardini et al. (27) reported that atropine at very low doses induces central antinociception in rodents through an enhancement of cholinergic transmission. Soon after, it was discovered that the R-(+)-enantiomer of atropine, R-(+)-hyoscyamine, was responsible for the antinociceptive activity of the racemate, while the S-(–)-enantiomer, S-(–)-hyoscyamine, was devoid of any antinociceptive action (29). R-(+)-hyoscyamine, in the same range of analgesic doses, was also able to prevent amnesia induced by antimuscarinic drugs (35). It is interesting to note that this antinociceptive activity, different from that produced by direct muscarinic agonists and cholinesterase inhibitors, was not accompanied by typical cholinergic symptoms (e.g., tremors, sialorrhea, diarrhea, rhinorrhea, lacrimation). An investigation of the antinociceptive and anti-amnesic effect of atropine has demonstrated, using microdialysis techniques, that R-(+)-hyoscyamine, at cholinomimetic doses, produced an increase in acetylcholine (ACh) release from the rat cerebral cortex *in vivo*, indicating that it acts via a presynaptic mechanism (35).

On this basis, a synthetic program to modify the chemical structure of atropine was started, which aimed to develop cholinergic amplifiers endowed with more intensive antinociceptive and anti-amnesic activities than atropine but, like atropine, lacking cholinergic side effects. These compounds would, therefore, be potentially useful as analgesics and/or in pathological conditions characterized by cholinergic deficits (e.g., Alzheimer's

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Fig. 1. Chemical structure and synthesis of PG-9.

disease). Of the many compounds synthesized and studied, α -tropanyl 2-(4-bromophenyl)propionate labeled PG-9 (45) (Fig. 1) showed an interesting pharmacological profile.

CHEMISTRY

The chemical structure of the lead compound, R-(+)-hyoscyamine, was extensively manipulated in order to overcome the stability problems associated with tropane acid derivatives and to obtain more efficacious antinociceptive compounds. The removal of the hydroxyl group to form α -tropanyl 2-phenylpropionate (ET-103) (43) gave a compound 70-times less potent but with similar efficacy as the lead compound (45). Better results were obtained with the series of substituted 2-phenylpropionic acid esters; in fact, with the appropriate substituent on the phenyl ring (4-Br, 4-F, 4-OMe, 2-, 3-, or 4-Cl) compounds which showed lower potency than ET-103 but a much higher analgesic efficacy were obtained (45). In this series, as well as in that of the 2-phenoxypropionic acid derivatives, antinociceptive activity was limited to the α -tropanyl ester (44). Among all compounds of the series, α -tropanyl 2-(4-bromophenyl)propionate (PG-9) showed the best combination of potency and efficacy and was selected for further studies. The synthetic pathway used for its synthesis is shown in Fig. 1. Single enantiomers were synthesized and studied because PG-9 possesses a stereogenic center (68,39). They showed enantioselectivity in pharmacological activity, although, unlike atropine enantiomers (35), both stereoisomers of PG-9 had analgesic and nootropic properties. In any case, the most potent and efficacious stereoisomer (S-(+)-PG-9) possesses the same spatial arrangement at the chiral center as the lead compound, R-(+)-hyoscyamine (68).

IN VIVO STUDIES

Antinociceptive Properties

PG-9 induced antinociception in mice and rats. Antinociception was elicited, regardless of the noxious stimulus used: thermal (hot-plate and tail flick tests), chemical

Fig. 2. Dose-response curves of PG-9 i.p. (a) and i.c.v. (c) in the mouse hot-plate test. The PG-9 time course of 40 mg/kg i.p. is reported in b and 20 µg per mouse i.c.v. in d from the same test. Vertical lines give $\pm$ S.E.M.. Each point is the mean of at least 10 mice. $^{\wedge}P < 0.05$; $^*P < 0.01$ vs. controls. In a and c PG-9 was administered 15 min before the test.

(abdominal constriction test) or mechanical (paw pressure test). The methods used were those of O'Callaghan and Holtzman (62), D'Amour and Smith (16), Koster et al. (53), and Leighton et al. (56), respectively.

In the mice hot-plate test PG-9, by systemic administration (i.e., i.p., p.o., i.v.), produced a dose-dependent increase in the pain threshold (Fig. 2; ref. 37). A similar effect was obtained with the abdominal constriction test in mice (Fig. 3, panel A). The maximal

antinociceptive effect of PG-9 was reached at 15 min after its administration. Thereafter, the effect slowly diminished (Fig. 2, panels B and D). PG-9 also produced an increase in

Fig. 3. Antinociceptive effect of PG-9 and antagonism by hemicholinium-3 (HC-3) (1 μ g per mouse i.c.v.), atropine (5 mg/kg i.p.), and dicyclomine (10 mg per mouse i.p.) on the enhancement of pain threshold induced by PG-9 (20 mg/kg s.c.) in the mouse abdominal constriction test induced by 0.6% acetic acid (a) and in the rat paw-pressure tests (b). HC-3, atropine or dicyclomine were injected, respectively, 5 h, 15 min and 10 min before testing. In the abdominal constriction test the nociceptive responses were recorded 15 min after PG-9 administration. Vertical lines show S.E.M. [^] $P < 0.05$; * $P < 0.01$ vs. saline controls. ^o $P < 0.01$ vs. PG-9 (20 mg/kg s.c.). Numbers inside the columns indicate the number of mice or rats.

the pain threshold in rats (paw pressure: Fig. 3, panel B; tail flick: ref. 37) with a pharmacological profile similar to that in mice. Both PG-9 enantiomers dose-dependently increased the pain threshold in mouse hot-plate and abdominal constriction test, although R-(+)-PG-9 was slightly more effective than S-(–)-PG-9 (37,68).

PG-9 is endowed with central antinociceptive activity. It was, in fact, possible to reach the same intensity of analgesia by injecting PG-9 directly into the cerebral ventricles by methods previously described (46) at 10–30 µg per mouse (Fig. 2, panel C; ref. 37). Its activity at such low doses ruled out the possibility that the antinociception could have been due to retrodiffusion of the drug from the cerebral ventricles to the periphery.

PG-9 showed good antinociceptive efficacy in comparison to R-(+)-hyoscyamine or well known analgesic drugs, such as morphine, diphenhydramine, or clomipramine. As a matter of fact, a comparison of the areas under the activity curves of the above-mentioned compounds at the highest doses that do not impair normal behavior of mice revealed that PG-9 was as effective as morphine and more effective than R-(+)-hyoscyamine, diphenhydramine, or clomipramine (37).

At doses lower than 1 mg/kg, PG-9 reduced the number of abdominal constrictions induced by i.p. injection of 0.3% acetic acid and reversed hyperalgesia induced by morphine withdrawal.

PG-9 antinociception is not due to an antiinflammatory action. At concentrations up to 10^{–4} mol/L PG-9 did not inhibit inducible COX activity, while indomethacin (IC₅₀) 27 × 10^{–6} mol/L was effective. Furthermore, PG-9 failed to suppress carrageenan-induced paw edema at analgesic doses (Table 1).

Antiamnesic Activity

PG-9 ameliorates impaired cognitive processes in mice. In the passive avoidance test this compound prevented amnesia induced by scopolamine, dicyclomine (Fig. 4), or (–)-ET-126 (36). Complete prevention of amnesia was obtained with PG-9 at 10 mg/kg i.p. (Fig. 4) or 20 µg i.c.v. (36). At these doses PG-9 had only weak analgesic activity in the hot-plate test. To prevent amnesia, PG-9 was administered at 20 min before the training session, since the time-course of the antiamnesic activity of PG-9 indicates that the compound reaches its maximal effect at 15–30 min after injection.

TABLE 1. Effect of PG-9 on carrageenan-induced paw edema in rats

Pretreatment	Treatment	Dose (mg/kg i.p.)	Paw volume (ml ± S.E.M.)
Saline	Saline		1.25 ± 0.08
Carrageenan	Saline		2.31 ± 0.06
Carrageenan	PG-9	20	2.37 ± 0.08
Carrageenan	PG-9	40	2.39 ± 0.10
Carrageenan	Indomethacin	1	1.45 ± 0.07*

Indomethacin was used as positive control; *n* = 5 rats per group. **P* < 0.05 in comparison with carrageenan-saline controls. PG-9 was injected 15 min before the test.

Fig. 4. Effect of PG-9 (i.p.), aniracetam (p.o.), and physostigmine (i.p.) on dicyclomine-induced amnesia in mouse passive avoidance test and, under the same experimental conditions, effect of PG-9 on scopolamine amnesia. Punishment consists of a fall into cold water (10°C). PG-9, aniracetam, and physostigmine were injected 20 min before the training session. Scopolamine and dicyclomine were injected immediately after the training session. * $P < 0.01$ vs. saline controls. Each column represents the mean of at least 25 mice.

In the passive avoidance test, an improvement in cognition of animals with no memory impairment is difficult to demonstrate. As a matter of fact, PG-9 as well as well-known nootropic drugs, such as piracetam, aniracetam, and rolipram or cholinomimetics, such as physostigmine and oxotremorine, do not show any memory facilitation in normal animals (15,41). The procognitive activity of PG-9 was unmasked by using a social learning test, which was performed according to methods described by Mondadori et al. (61). PG-9 exerted beneficial effects on cognitive performance by prolonging the time normally required by rats to delete mnemonic information (data not shown).

Effect of PG-9 on Animal Behavior

PG-9 produced its maximal antinociceptive effect at 40 mg/kg s.c. without any visible modification in gross behavior of either mice or rats. Moreover, mice treated with the same dose of PG-9 retained motor coordination in the rotating rod test (method of Kuribara et al., 54) (Table 2). Under these experimental conditions, the effects of PG-9 were compared to those of pilocarpine or physostigmine at equi-effective doses (Table 2). Both the muscarinic agonist and the inhibitor of cholinesterase produced a statistically significant reduction in endurance time on the rotating rod. The spontaneous motility of mice,

TABLE 2. Effect of PG-9 in comparison with pilocarpine and physostigmine in the rotarod test

Treatment s.c.	Endurance time on rotarod (sec)			
	Before treatment	After treatment		
		15 min	30 min	45 min
Saline	104.3 ± 6.2 (18)	106.7 ± 6.0 (18)	107.8 ± 5.9 (18)	103.5 ± 6.2 (18)
PG-9 (40 mg/kg)	101.2 ± 5.9 (10)	96.7 ± 8.2 (10)	95.6 ± 9.4 (10)	106.2 ± 8.4 (10)
Pilocarpine (10 mg/kg)	108.2 ± 8.4 (11)	66.5 ± 7.1* (11)	61.3 ± 9.6* (11)	74.4 ± 8.7* (11)
Physostigmine (200 µg/kg)	93.4 ± 5.7 (9)	61.4 ± 6.8* (9)	54.4 ± 8.1* (9)	52.3 ± 8.8* (9)

* $P < 0.05$ vs. saline controls. The number of mice is shown in parentheses.

evaluated by the Animex apparatus, as well as the exploratory behavior, studied by the hole-board test (36), were normal after the administration of PG-9 at 40 mg/kg s.c. or 30 µg per mouse i.c.v. Impaired motor coordination and spontaneous motility were observed in mice after PG-9 was administered alone at doses starting at 100 mg/kg s.c., whereas its LD₅₀ was 400 mg/kg s.c.

Effect on Intestinal Motility

PG-9 did not modify intestinal transit time in mice at analgesic and anti-amnesic doses as determined by the technique of Reynell and Spray (67) (data not shown). In contrast, other analgesic drugs, such as morphine, significantly retarded gastrointestinal propulsion, whereas the cholinesterase inhibitor, neostigmine, accelerated net propulsion (72). The lack of effect of PG-9 on intestinal motility indicates that this compound has the same analgesic activity and may be superior to opioid analgesics, which produce constipation, or to classical cholinomimetics, which cause diarrhea.

IN VITRO STUDIES

Effect on Endogenous Nerve Growth Factor

In vitro administration of PG-9 increased the secretion of Nerve Growth Factor (NGF) by astrocytes in a dose-dependent manner (36). After addition of PG-9 the maximal NGF levels were 17.6 times greater than the control value. In cultured astrocytes, effective concentrations of PG-9 produced no morphological changes. However, slight cell toxicity or morphological changes were observed with PG-9 at 1 mg/mL. (36). Cultured quiescent astrocytes can be used to study the effects of drugs on NGF synthesis since NGF synthesis is regulated in a growth-dependent manner in cultured astrocytes; most of the astrocytes in the brain are in the quiescent phase and do not express the NGF gene with *in vivo* administration.

Fig. 5. Dose-response curves of PG-9 on nicotine (4 μ M) (●) and electrically (0.1 Hz; 0.5 msec; double threshold voltage) evoked contractions (○) of guinea-pig ileum myenteric plexus longitudinal muscle strip expressed as percentage variation of contractions. Each point represents the mean of at least 6 experiments and vertical lines give S.E.M. * $P < 0.05$ calculated in the range between 0.1 pM–0.1 nM.

Effect on Isolated Guinea Pig Ileum

When added to the organ bath at concentrations ranging from 1 pmol/L to 0.1 nmol/L, PG-9 potentiated contractions evoked by either nicotine (4 μ mol/L) or electrical stimulation [0.1 Hz, 0.5 msec, voltage double threshold, method of Paton and Vizi (64)] (Fig. 5). As measured by the area under the curve, PG-9 potentiated contractions induced by nicotine to a greater extent than those induced by electrical stimulation. The potentiation was no longer observed when the concentration of PG-9 in the medium reached 0.1 nM. At 10 nmol/L PG-9 began to inhibit both types of evoked contractions. Nicotine-evoked contractions were about four times larger than those evoked electrically (Fig. 5). The difference in the magnitude of contraction is probably due to the fact that during electrically-evoked contractions both intramural cholinergic and sympathetic fibers are activated, whereas only cholinergic fibers are activated during nicotine-evoked contractions. Norepinephrine released during electrical stimulation is likely to limit the effect of ACh released after the administration of low concentrations of PG-9. The greater potentiation of the nicotine-evoked contractions compared to those elicited by electrical stimulation can be explained by the inhibitory effect of norepinephrine, which is released only during electrical stimulation (27).

Fig. 6. Dose-response curves of PG-9: (a) on electrically evoked phrenic nerve-hemidiaphragm contractions (0.2 Hz, 0.5 msec, twice threshold voltage); (b) on tritium efflux from the same preparation preloaded with [³H]choline. Each point represents the mean of 6 experiments. Vertical lines give $\pm$ S.E.M. * $P < 0.05$ calculated in the range between 10^{-9} – 10^{-12} M.

Rat Phrenic Nerve-Hemidiaphragm Preparation

PG-9 (1 pmol/L – 1 nmol/L; $> 1 \mu\text{mol/L}$) potentiated contractions of hemidiaphragm evoked by electrical stimulation of the left phrenic nerve (Fig. 6, panel A). ACh release (Fig. 6, panel B), studied by the method of Bülbring (9) as modified by Wessler and Kilbinger (75), was also enhanced by PG-9, while contractions evoked by the direct stimulation of the diaphragm were not modified by the drug. At concentration greater than $1 \mu\text{mol/L}$, numerous muscarinic antagonists, such as atropine, pirenzepine, dicyclomine, or glycopyrrolate are known to enhance hemidiaphragm contractions by blocking musca-

rinic autoreceptors (66,76). The effects of high concentrations of PG-9, may, therefore, involve an antagonism at muscarinic receptor subtypes. The potentiating effect of PG-9 (1 pmol/L to 0.1 nmol/L) on both parameters parallels the effect of R-(+)-hyoscyamine under the same experimental conditions (33), although the underlying mechanism of PG-9 action should still be considered unknown. The inability to inhibit hemidiaphragm contractions induced by electrical stimulation rules out the possibility that PG-9 may act as a local anesthetic. Local anesthetics, such as lidocaine and procaine, dose-dependently inhibit the electrically-induced contractions of this preparation and abolish them at higher concentrations (1). Also, unlike local anesthetics, PG-9 did not inhibit cutaneous muscle reflex in guinea pig dorsal skin.

MECHANISM OF ACTION

The antinociceptive effect of PG-9 appears to depend on cholinergic activation since it is antagonized by the muscarinic antagonist atropine (Fig. 3, panels A and B; ref. 37), the M_1 antagonists dicyclomine (Fig. 3, panels A and B) and pirenzepine (37), as well as by the ACh depletor HC-3 (Fig. 3, panels A and B). Moreover, the antagonism of PG-9-induced antinociception by i.c.v. injected HC-3 in mice (see *Antinociceptive Properties* section), indicates that the site of action of PG-9 is central.

Microdialysis studies (method of Giovannini et al., 39) indicate that PG-9 increases ACh release from the rat cerebral cortex; this effect peaked at 45–60 min after administration of the drug, and the ACh levels returned to basal values within 120 min (Fig. 7). PG-9 enhanced ACh release at the same dose range (10–20 mg/kg, i.p.) that PG-9 exerts its antinociceptive and anti-amnesic activities. The latency for the maximal potentiation of ACh release was longer than for drug activity, possibly because ACh may require time to diffuse from the synaptic cleft to the microdialysis tube. The hypothesis that PG-9 acts via a presynaptic cholinergic mechanism is supported by the following observations: PG-9 enhances ACh release and contractions of the rat hemidiaphragm preparation (Fig. 6); PG-9 potentiates electrically and chemically evoked contractions of guinea pig ileum longitudinal muscle strips (Fig. 5) without modifying their basal tone; and the ACh depletor HC-3 antagonizes PG-9-induced antinociception (Fig. 3).

A postsynaptic mechanism of action for PG-9 can be ruled out since, as reported by Bartolini et al. (3,5), HC-3 was unable to antagonize antinociception induced by postsynaptic muscarinic receptor agonists such as oxotremorine, McN-A-343, or AF-102B. Moreover, PG-9 did not elicit the typical cholinergic symptoms (e.g., tremors, sialorrhea, diarrhea, rhinorrhea, lacrimation) produced by directly acting postsynaptic muscarinic agonists (8).

It is well known that the activation of the nicotinic system induces antinociception. PG-9, even though it increases the extracellular levels of ACh, enhances the pain threshold, an effect not prevented by mecamylamine. Thus, PG-9 does not act via nicotinic receptor-mediated mechanism of action. This hypothesis is supported also by the fact that muscarinic antagonists do not prevent nicotinic antinociception at doses that antagonize muscarinic antinociception (29).

It has long been known that the activation of the cholinergic system induces antinociception (12,26,47–49,57,65) and facilitates cognitive processes (15). It is, therefore, con-

Fig. 7. Dose-response curve of PG-9 on ACh release from parietal cortex. All values are expressed as changes over basal output. PG-9 was administered at 60 min as shown by the arrow. Vertical lines give $\pm$ S.E.M. Each point represents the mean of at least 5 independent experiments. Doses of PG-9 are expressed as mg/kg i.p. Significant differences were evaluated by comparing the percentage variation vs. the mean $\pm$ S.E.M. of all predrug determinations. * $P < 0.05$ vs. controls.

ceivable that enhancement of extracellular levels of ACh may be considered responsible for the antinociceptive effect of PG-9. Moreover, the PG-9-induced potentiation of endogenous ACh release may also counteract the amnesic effect produced by the antimuscarinic drugs scopolamine and dicyclomine.

ACh release can be increased by blocking M_2/M_4 muscarinic autoreceptors (55,59,73,71). The affinity profile of PG-9 versus M_1 [rabbit vas deferens, according to the methods of Eltze (23) as modified by Dei et al. (17)], M_2 [guinea pig atrium, according to the methods of Eltze et al. (21) modified by Dei et al. (17)], M_3 [guinea pig ileum, according to the methods of Eltze and Figala (22)] and putative M_4 receptors [prepuberal guinea pig uterus, according to the methods of Dörje et al. (19)] shows low M_4/M_1 (11.2) and M_2/M_1 selectivity (8.3) ratios (Table 3). In this study the selectivity of PG-9 was compared to that of the M_4 antagonist R-(+)-hyoscyamine (30) and M_2 antagonist AFDX-116 (38). It is possible that a low selectivity ratio of 11.2 may be high enough to enhance the pain threshold and to reverse amnesia as a consequence of ACh release. The M_2 muscarinic antagonists AFDX-116 (38), methoctramine (60), and AQRA-741 (18), as well as PG-9, all of which have cholinergic presynaptic antinociceptive (4,28,42) and antiamnesic (2) properties and are capable of increasing ACh release (55,73), have a selectivity ratio M_2/M_1 comparable to that of PG-9. Binding studies performed on the m_1 – m_4 human

muscarinic receptor subtypes expressed in CHO cells (11,20) confirm the results obtained by functional studies (Table 3). It cannot be excluded, however, that other mechanisms capable of potentiating the endogenous cholinergic system may also be involved in the antinociceptive and anti-amnesic effects of PG-9.

It has been demonstrated that D₂ dopaminergic (40,51,70,74), A₁ adenosinergic (10,52), H₃ histaminergic (13), 5-HT₄ serotonergic heteroreceptors (14), and 5-HT_{1A} receptors (7) increase ACh release. However, the above-mentioned receptors are not involved in a PG-9 mechanism of action. In fact, PG-9 is able to interact with D₂, H₃, 5-HT₄, and 5-HT_{1A} receptors only at concentrations higher than 10⁻⁶ M as revealed by binding studies (data not shown). These results are supported by the fact that quinpirole (D₂ agonist), N⁶-cyclopentyladenosine (A₁ agonist), R-(α)-methylhistamine (H₃ agonist), GR-48125 (5-HT₄ antagonist), and NAN 190 (5-HT_{1A} antagonist), at doses able to prevent the antinociception induced respectively by haloperidol (29), caffeine (34), thio-peramide (58), BIMU 1 and BIMU 8 (32), and 5-HT_{1A} agonists (25,31), failed to prevent PG-9 antinociception (37).

Neurotransmitter systems, other than the cholinergic, are not involved in PG-9 antinociception. At concentrations higher than 10⁻⁶ M this compound interacts with the following receptors, channels or receptor subtypes: α_1 , α_2 , β_1 , β_2 adrenoceptors, D₁, GABA_A, GABA_B, H₁, NK₁, δ , κ , μ opioid, 5-HT_{1D}, 5-HT₂, 5-HT₃, and K⁺ channels: ATP-sensitive K⁺ channel, voltage-dependent K⁺ channel, and Ca²⁺-activated K⁺ channel. The inability of the opioid antagonist naloxone, the GABA_B antagonist CGP-35348, and the biogenic amine depletor reserpine (37) to prevent PG-9 antinociception is in agreement with the binding data. Pretreatment with pertussis toxin (PTX) prevented opioid (63), catecholaminergic, GABAergic (50), histaminergic (24), and purinergic (69) analgesia but not muscarinic antinociception (24). Since PG-9 antinociception was not prevented by pretreatment with PTX (6), the hypothesis that a cholinergic mechanism underlies the PG-9 activity is further supported.

TABLE 3. Affinity profiles of PG-9, R-(+)-hyoscyamine and AFDX-116 at M₁–M₄ muscarinic receptors and binding affinities of PG-9 and AFDX-116 for m₁–m₄ muscarinic receptor subtypes expressed in Chinese hamster ovary cells (CHO-K1)

	pA ₂ Values			
	M ₁ rabbit vas deferens	M ₂ rat left atrium	M ₃ rat ileum	M ₄ -putative guinea-pig uterus
PG-9	6.69 ± 0.04	6.81 ± 0.09	6.86 ± 0.10	7.74 ± 0.05
R-(+)-hyoscyamine	7.05 ± 0.05 ^a	7.25 ± 0.04 ^a	6.88 ± 0.05 ^a	9.56 ± 0.01 ^a
AFDX-116	6.85 ± 0.14 ^b	7.12 ± 0.11 ^b	6.34 ± 0.13 ^c	6.70 ± 0.06
	pK _i Values			
	m ₁	m ₂	m ₃	m ₄
PG-9	6.23 ± 0.05 ^d	6.31 ± 0.06 ^d	6.19 ± 0.11 ^d	7.10 ± 0.09 ^d
AFDX-116	6.84 ± 0.14 ^b	7.12 ± 0.11 ^b	6.34 ± 0.13 ^b	6.70 ± 0.06

Each value represents the mean ± S.E.M.; ^a ref. 30; ^b ref. 23; ^c ref. 22; ^d Ghelardini et al. *Arzneimittelforschung* 1999;49:483.

SUMMARY

PG-9 is a 2-arylpropionic acid ester, structurally related to atropine. It has central antinociceptive and antiamnesic effects in mice and rats. These effects are exerted without impairment of motor coordination and without typical cholinergic symptomatology. PG-9 potentiates evoked contractions of smooth and striated muscle. The mechanism of PG-9 action in the central and peripheral nervous systems appears to involve potentiation of endogenous cholinergic activity and enhancement of extracellular ACh levels. Moreover, *in vitro* administration of PG-9 increases secretion of NGF by astrocytes in a concentration-dependent manner.

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