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Enhancement of adenosine-induced relaxant responses of the guinea-pig isolated taenia coli and aortae by a novel nootropic agent BY-1949: comparison with dipyridamole

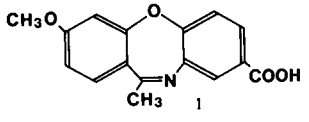
SHIGERU TANABE, YUGO IKEDA, YASUYUKI SHIRAKI, KAZUSHIGE SAKAI, *Department of Pharmacology, Exploratory Research Laboratories, Chugai Pharmaceutical Co., Ltd, Komakado, Gotemba, Shizuoka 412, Japan*

Abstract—In muscle strips of guinea-pig taenia coli and aortae, the effects of 3-methoxy-11-methyldibenz[b,f][1,4]oxazepine-8-carboxylate (BY-1949) on the relaxant response to adenosine have been compared with those of dipyridamole. BY-1949 (3×10^{-5}–10^{-4} M) as well as dipyridamole (3×10^{-7}–10^{-5} M) resulted in a significant potentiation of relaxant responses to a cumulative administration of adenosine (10^{-6}–10^{-3} M), but the drugs did not affect the response to noradrenaline and acetylcholine. The results indicate that BY-1949 exerts a selective potentiating effect on reactivity to exogenous adenosine.

Although impaired memory process is an extremely complex disease state in view of the poorly known pathogenesis, it is assumed that a reduction of the energy supply to the brain, such as cerebral hypoxia or ischaemia, leads to amnesia or learning deficits (Bass 1985; Jackson 1986; Ananth 1987). However, there has been no confirmed treatment for improving the cognitive disorders associated with organic brain syndromes.

A series of dibenzoxazepine derivatives has been synthesized and tested to find ideal therapeutic agents that improve cognitive function. Among these compounds, BY-1949 (I) improved retardation of the acquisition process on Sidman avoidance task in aged rats (Tanabe et al 1986). Furthermore, this compound increased the regional cerebral blood flow in conscious cats (Sugawa et al 1986) and improved the lowered cerebral glucose utilization in aged rats (Itoh et al 1987).

The aim of the present experiment in guinea-pig isolated taenia coli and aorta preparations was to examine some profiles of muscle relaxation induced by BY-1949.



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Materials and methods

The experiments were performed on strips of taenia coli and ring segments (2–3 mm length) of the thoracic aorta of male Hartley strain guinea-pigs (450–750 g). The muscle segments were suspended under a load of 1 g in a 10 mL organ bath containing a modified Krebs-Henseleit bicarbonate solution composed of: (mM) NaCl 119·0, KCl 4·8, $MgSO_4$ 1·2, KH_2PO_4 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 24·8, and glucose 10·0 (ascorbic acid 57 μM if noradrenaline was used). The bath was aerated thoroughly with a gas mixture of 95% O_2–5% CO_2. The pH of the gassed solution was 7·4 at 37°C. The strips of taenia coli were connected to an isotonic transducer (ME Commercial Co., ME-4012) for continuous recording of isotonic tension. The segments of thoracic aortae were connected to a Nihon Kohden force transducer (SB-1T) for continuous recording of isometric tension. Recordings were made on a Yokogawa self-balancing potentiometric recorder (model 3066).

Four preparations were run concurrently. The muscle segments were equilibrated for at least 2 h, with washes every 20 min, before exposure to drugs. The drugs were added to the 10 mL organ bath in a volume of 0·1 mL. Subsequent doses of agents, such as adenosine, noradrenaline and acetylcholine, were increased by a factor of about 3, and introduced when the preceding ones had reached a steady value. Thus, a cumulative concentration-percentage maximal response curve to an agent was constructed for each tissue in the absence and the presence of BY-1949 or dipyridamole. BY-1949 or dipyridamole was added to the organ bath, and 2–5 min later the cumulative concentration-response curve to an agent was constructed for each tissue. All drug concentrations are expressed as final molar (M) concentrations in the bath solution.

Drugs. The drugs used were: 3-methoxy-11-methyldibenz[b,f][1,4] oxazepine-8-carboxylate (BY-1949, $C_6H_{13}NO_4$, mol. wt 283·28) (used as a sodium salt), adenosine (Sigma), dipyridamole (ampoule, C.H. Boehringer Sohn, Ingelheim), papaverine hydrochloride (Tokyo Kasei), (−)-propranolol hydrochloride (ICI), acetylcholine chloride (Daiichi Seiyaku) and (±)-noradrenaline hydrochloride (Sankyo). The drugs were dissolved in or diluted with 0·9% saline solution.

Statistical analysis. The data in the test are expressed as the mean ± s.e.m. Welch's *t*-test for unpaired observations was used for statistical evaluation of the data; $P<0.05$ was considered statistically significant.

Results

Taenia coli. BY-1949 (10^{-6}–10^{-3} M) and dipyridamole (10^{-6}–10^{-3} M) added cumulatively to the organ bath produced a concentration-dependent reduction of tone in the muscle strip preparations. Papaverine (10^{-4} M) yielded a maximum relaxation, which was taken as 100%. The EC50 (the concentration reducing the muscle tone by 50%) values of BY-1949 and dipyridamole were 3·82 ± 0·09 and 4·40 ± 0·14 (each n = 8), respectively. When compared on the EC50 values, BY-1949 was approximately four times less potent than dipyridamole in relaxing the taenia coli.

Adenosine or noradrenaline added to the organ bath induced reproducible muscle relaxation. Curve of cumulative concentration-response to adenosine (10^{-6}–10^{-3} M) was obtained in the absence and the presence of BY-1949 (3×10^{-5}–10^{-4} M) or dipyridamole (10^{-6}–10^{-5} M). As depicted in Fig. 1A, BY-1949 and dipyridamole caused a parallel shift to the left of the concentration-response curve to adenosine. However, the curve of cumulative concentration-response to noradrenaline (10^{-8}–10^{-5} M) virtually remained unaltered in the presence of BY-1949 (10^{-4} M) or dipyridamole (10^{-6} M). The EC50 values of

Correspondence and present address: K. Sakai, International Development Department, R & D Division, Chugai Pharmaceutical Co., Ltd., Kyobashi, Chuo-ku, Tokyo 104, Japan.

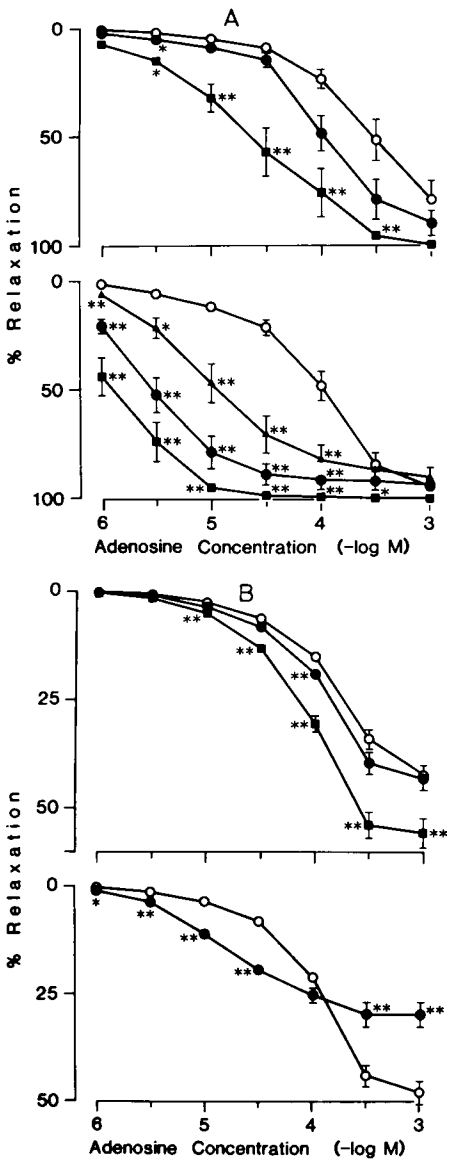

FIG. 1. Enhancement of the relaxant response to adenosine by BY-1949 (upper panels) and dipyridamole (Dip) (lower panels) in guinea-pig isolated A) taenia coli and B) aorta preparations. Each point represents the mean of 8 observations in 8 experiments. Vertical bars show the mean ± s.e.m. * $P<0.05$, ** $P<0.01$ compared with the corresponding control values. In aorta preparations, noradrenaline (4×10^{-6} M) yields a contraction of about 70–80% of the maximum, which was taken as 100%. Papaverine (10^{-4} M) yields a maximum relaxation, which was taken as 100%.
O, control A, upper: BY-1949 ● 3×10^{-5} M ■ 10^{-4} M. A, lower: dipyridamole ▲ 10^{-6} M, ● 3×10^{-6} M ■ 10^{-5} M. B, upper: as in A. B, lower: dipyridamole ● 3×10^{-7} M.

noradrenaline in the absence and the presence of BY-1949 were 6·29 ± 0·01 and 6·43 ± 0·09 (each n = 5, n.s.), respectively. The values in the absence and the presence of dipyridamole were 6·29 ± 0·01 and 6·28 ± 0·01 (each n = 5, n.s.), respectively.

Thoracic aorta. Sustained contractions were induced in the presence of a submaximal concentration of 4×10^{-6} M noradrenaline after preincubation for 20 min with 10^{-6} M propranolol. In the strips contracted with noradrenaline, the addition of BY-1949 (10^{-6}–10^{-4} M) or dipyridamole (10^{-7}–10^{-5} M) caused no significant relaxation (not shown).

In similar preparations, adenosine or acetylcholine added to the organ bath resulted in reproducible muscle relaxation. Curve of cumulative concentration-response to adenosine (10^{-6}–10^{-3} M) or acetylcholine (10^{-8}–10^{-5} M) was constructed in the absence and the presence of BY-1949 (3×10^{-5}–10^{-4} M) or dipyridamole (3×10^{-7} M). As shown in Fig. 1B, BY-1949, in the concentration used, significantly enhanced the relaxant response to adenosine. Dipyridamole was effective in augmenting the muscle relaxation induced by adenosine in concentrations of 10^{-6} to 10^{-4} M, but a cumulative concentration-response curve to acetylcholine (10^{-8}–10^{-5} M) was not significantly altered in the presence of BY-1949 (10^{-4} M) or dipyridamole (3×10^{-7} M). The EC15 values of acetylcholine in the absence and the presence of BY-1949 were 4·71 ± 0·16 and 4·65 ± 0·17 (each n = 5, n.s.), respectively. Also, the values in the absence and the presence of dipyridamole were 4·71 ± 0·16 and 4·15 ± 0·22 (each n = 5, n.s.) respectively.

Discussion

The present experiment demonstrated that BY-1949 significantly potentiated the relaxant response to adenosine in guinea-pig isolated taenia coli and aorta preparations, although dipyridamole failed to enhance the muscle relaxation induced by higher concentrations of adenosine in isolated aorta preparations. The potentiating effect of BY-1949 was characteristically specific for adenosine-induced response, in that responses to noradrenaline and acetylcholine were unaffected. Thus, BY-1949 possesses a selective action in potentiating the relaxant response to adenosine.

Berne (1963) suggested a possible role of adenosine in the autoregulation of coronary blood flow. Furthermore, it has also been proposed that adenosine plays an important role in regulating cerebral blood flow during hypoxic and hypercapnic episodes (Winn et al 1979; Busija & Heistad 1984; Phillis et al 1984, 1985). Actually, cerebral or myocardial hypoxia leads to the release of adenosine, which in turn produces a strong vasodilatation and an increase in cerebral or coronary blood flow (Berne 1980; Heistad et al 1981). Thus, the physiological role of adenosine on the peripheral circulation draws special interest in cardiovascular physiology and pharmacology.

There are many reports that the disappearance or inactivation of adenosine is ascribed to the decomposition by adenosine deaminase and the uptake of adenosine into the tissues. Generally, adenosine potentiators such as dipyridamole have an inhibitory action on adenosine uptake and deamination (Bunag et al 1964), through which the mode of action of the potentiation induced by the adenosine potentiators has been partly explained. A similar mechanism is expected for BY-1949, even though it remains to be elucidated.

In summary, BY-1949 posesses a selective activity in potentiating adenosine actions. However, it remains to be determined whether the potentiating effect is relevant to the physiological actions of BY-1949 in cerebrovascular regulation, particularly during anoxia and hypercapnia.

References

Ananth, J. (1987) Specific effects of cyclandelate on memory. Drugs 33 (Suppl. 2): 97–102

Bass, E. (1985) Cardiopulmonary arrest. Pathophysiology and neurologic complications. Ann. Intern. Med. 103: 920–927

Berne, R. M. (1963) Cardiac nucleotides in hypoxia: Possible role in regulation of coronary blood flow. Am. J. Physiol. 204: 317–322

Berne, R. M. (1980) The role of adenosine in the regulation of coronary blood flow. Circ. Res. 47: 807–813

Bunag, R. D., Douglas, C. R., Imai, S., Berne, R. M. (1964) Influence of a pyrimidopyrimidine derivative on deamination of adenosine by blood. Ibid. 15: 83–88

Busija, D. W., Heistad, D. D. (1984) Factors involved in the physiological regulation of the cerebral circulation. Rev. Physiol. Biochem. Pharmacol. 101: 161–211

Heistad, D. D., Marcus, M. L., Gourley, J. K., Busija, D. W. (1981) Effect of adenosine and dipyridamole on cerebral blood flow. Am. J. Physiol. 240: H775–780

Itoh, E., Kobayashi, M., Sugawa, M., Noda, Y. (1987) Age-related decrease in rat brain glucose metabolism and the ameliorating effect of BY-1949 on it. Jap. J. Pharmacol. 43 (Suppl.): 73

Jackson, A. C. (1986) Neurologic disorders associated with mitral valve prolapse. Can. J. Neurol. Sci. 13: 15–20

Phillis, J. W., Preston, G., DeLong, R. E. (1984) Effects of anoxia on cerebral blood flow in the rat brain: Evidence for a role of adenosine in autoregulation. J. Cereb. Blood Flow Metabol. 4: 586–592

Phillis, J. W., DeLong, R. E., Towner, J. K. (1985) Adenosine deaminase inhibitors enhance cerebral anoxic hyperemia in the rat. Ibid. 5: 295–299

Sugawa, M., Tanabe, S., Ikeda, Y., Noda, Y. (1986) Effects of dibenzoxazepines (BYs) on regional cerebral blood flow (rCBF) in conscious cats. Jap. J. Pharmacol. 40 (Suppl.): 119

Tanabe, S., Ikeda, Y., Sakai, K. (1986) Conditioned avoidance learning in aged rats and the effects of the two dibenzoxazepines (BYs). Ibid. 40 (Suppl): 145

Winn, H. R., Rubio, R., Berne, R. M. (1979) Brain adenosine production in the rat during 60 seconds of ischemia. Circ. Res. 45: 486–492

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Brain penetration of orally administered sodium pyroglutamate

J. L. CHANAL, M. AUDRAN, M. T. SICARD, M. BRILEY*, *Faculty of Pharmacy, University of Montpellier, Avenue Charles Flahault, 34060 Montpellier, *Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France*

Abstract—The absorption and brain penetration of [^{3}H]pyroglutamate was determined after oral administration to rats. Gas-liquid chromatography of the methylated derivatives followed by mass fragmentometry was used to analyse the plasma and brain levels of pyroglutamate. [^{3}H]Pyroglutamate was separated from other labelled compounds by thin layer chromatography. The administration of 500 mg kg^{-1} [^{3}H]pyroglutamate resulted in a 30-fold increase in plasma levels and a doubling in the brain levels. Over 60% of the cerebral radioactivity was present as [^{3}H]pyroglutamate demonstrating that pyroglutamate is not only well absorbed but also penetrates in significant amounts into the brain.

Pyroglutamic acid (5-oxo-l-proline, 2-pyrrolidone-5-carboxylic acid) is found as the free amino acid in a number of tissues and body fluids (Wilk & Orlowski 1975) and as the *N*-terminal amino acid of various biological active peptides. In addition to its transformation to glutamate by 5-oxo-prolinase, pyroglutamate has intrinsic activity at central glutamate receptors (Dusticier et al 1985) as an agonist (Rieke et al 1984; Antonelli et al 1984) although some antagonist activities have also been reported (Van Harreveld & Fifkova 1971; Continho-Netto et al 1981). A recent report also suggests that it may improve performance in rats in a passive avoidance memory retention task (Drago et al 1987).

Oral administration of pyroglutamate to rats has been shown to increase the endogenous levels of brain pyroglutamate and glutamate (Caccia et al 1982), although whether this was by accumulation from the plasma or an indirect stimulation of the synthesis of glutamate and subsequently pyroglutamate in the brain was not demonstrated. In view of the increasing interest in excitatory amino acids (for recent review see Trends in Neurosciences Special Issue 1987), pyroglutamate could prove to be a useful research tool as one of the fairly rare orally active glutamate receptor agonists.

We demonstrate here that orally administered [^{3}H]pyroglutamate penetrates freely into the brain where a substantial proportion of it remains for several hours as the unaltered drug and that it is subsequently metabolized to glutamate.

Correspondence to: M. Briley, Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France.

Materials and methods

Materials. [^{3}H]Pyroglutamate, obtained from the Commission d'Energie Atomique (Saclay, France), had a specific activity of 85 Ci mmol^{-1}. *N*-Ethyl pyroglutamate and sodium pyroglutamate was synthesized by Pierre Fabre Research Centre, Castres, France.

Drug administration and sampling. Male Wistar rats (200–220 g), fasted for 18 h with free access to water, were administered orally sodium pyroglutamate (500 mg kg^{-1}) containing sodium [^{3}H]pyroglutamate (20 μCi/rat) as a suspension in 5% aqueous gum arabic.

Animals were killed by exsanguination through cardiac puncture 15, 30, 60, 90, 120, 240 and 480 min after drug administration. The plasma was recovered by centrifugation for 10 min at 3500 rev min^{-1} and the brains rapidly removed, frozen, and stored at −30°C.

Extraction and gas-liquid chromatography/mass spectrometric analysis. The frozen brains were homogenized in 80% aqueous ethanol (0·1 g tissue mL^{-1}) and the radioactivity of an aliquot equivalent to approximately 100 μg of brain determined in a Packard TriCarb 3255 scintillation counter. Aliquots (0·1 mL) of plasma were similarly extracted with 0·9 mL 80% aqueous ethanol. To 1 mL of each of the alcoholic suspensions of brain and plasma was added 0·1 mL of a solution of 80 μg mL^{-1} sodium *N*-ethylpyroglutamate as an internal standard for mass fragmentometry.

Ethanolic extracts of two brain samples and two plasma samples were left to stand for 30 min at 4°C before centrifuging at 3000 rev min^{-1} for 5 min. The supernatants were treated as described by Marstein et al (1973) namely, acidification to pH 1·5–2 with 6 M HCl, passage through a 0·7 × 6 cm Dowex 50W X-4 column to remove the free amino acids and amines, elution with 4 mL water-methanol (1:3 v/v) and finally lyophilization. The lyophilized extracts were methylated by the addition of 0·2 mL methanolic hydrochloric acid (prepared by adding 1 mL acetyl chlorate dropwise to 4 mL methanol). After 5 min at room temperature, the samples were evaporated to dryness.

The methyl ester residues were taken up in 100 μL ethanol and