Effects of Pantoyl-GABA on GABA\textsubscript{A} and GABA\textsubscript{B} Receptors in the Rat Brain

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Abstract—The interactions of the "antidementia drug" pantoyl-\(\gamma\)-aminobutyric acid (pantoyl-GABA) with \(\gamma\)-aminobutyric acid (GABA) receptors were investigated by studies on bindings of radiolabelled ligands in rat brain. Pantoyl-GABA inhibited the binding of \([^{3}\text{H}]\text{GABA}\) to GABA\textsubscript{A} receptors and those of \([^{3}\text{H}]\text{baclofen}\) and \([^{3}\text{H}]\text{GABA}\) to GABA\textsubscript{B} receptors in the rat cerebral cortex. These data suggest that pantoyl-GABA interacts with both types of GABA receptors in the rat brain.

Recent evidence has indicated the existence of two types of GABA receptors. One difference between these types is in their sensitivity to bicuculline (1, 2). Conventional bicuculline-sensitive sites are linked to Cl\textsuperscript{-} channels and interact with barbiturate- and benzodiazepine-recognition sites (3). These are pharmacologically distinct from bicuculline-insensitive sites, termed GABA\textsubscript{B} receptors, for which baclofen is a selective agonist of these sites (4, 5).

Dysfunction in the central cholinergic system has been thought to be a major cause of Alzheimer’s disease (6–8). The calcium salt of pantoyl-GABA has been used clinically as an antidementia drug in Japan (9). In vitro, it has been found to increase high affinity uptake of choline by slices of rat brain and their release of ACh, suggesting that it facilitates central cholinergic function (10, 11).

Pantoyl-GABA consists of GABA and pantoyl moieties, and it is an agonist for GABA\textsubscript{A} sites, and the increase of ACh release from brain slices caused by pantoyl-GABA is antagonized by bicuculline (11). Thus, the interaction of pantoyl-GABA with GABA receptors is related to its pharmacological action as an antidementia drug. The effect of pantoyl-GABA on GABA\textsubscript{B} sites, however, has not yet been studied. In this work, we examined the effects of pantoyl-GABA on the two types of GABA receptors by experiments on its influence on radiolabelled ligand bindings to membranes from rat brain.

Male Sprague-Dawley rats were decapitated and the cerebral cortex was removed and homogenized in 10 volumes of ice-cold 0.32 M sucrose. The homogenate was centrifuged at 1,000 g for 10 min, and the supernatant was collected and recentrifuged at 10,500 g for 30 min. The resulting pellet was dispersed in distilled water and centrifuged at 100,000 g for 10 min. The final pellet was stored at \(-80^\circ\text{C}\) overnight. The frozen membranes were then thawed and suspended in 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 100,000 g for 10 min. This procedure was repeated twice more, and the final pellet was suspended in the above buffer.

For assay of \([^{3}\text{H}]\text{GABA}\) binding to GABA\textsubscript{A} sites in displacement studies, the membrane
suspension was incubated at 25°C for 10 min in 1 ml of medium containing 50 mM Tris-HCl buffer (pH 7.4), 2.5 mM CaCl₂, 10 nM [³H]GABA and 100 μM bicuculline with drugs at the indicated concentrations. The procedure for assay of [³H]baclofen binding was the same as that for assay of [³H]GABA binding except that 20 nM [³H]baclofen was used instead of [³H]GABA and bicuculline was omitted from the medium. For determination of nonspecific binding, 100 μM baclofen was added to the incubation mixture in both assays. For assay of [³H]GABA binding to GABAA sites, the membrane suspension was incubated at 25°C for 10 min in 1 ml of medium containing 50 mM Tris-HCl buffer (pH 7.4) and 5 nM [³H]GABA. GABA (1 mM) was used to determine nonspecific binding. The reaction was terminated by filtering the incubation mixture through a glass filter (Whatman GF/F). The filter was then washed 3 times with 2 ml of ice-cold Tris-HCl buffer, and its radioactivity was counted in a liquid scintillation counter. Specific binding was defined as total binding minus nonspecific binding.

When CaCl₂ (2.5 mM) is present in the incubation mixture, [³H]GABA binds to GABAA sites as well as GABAA sites (12). For the GABAA binding assay, bicuculline (100 μM) was included in the medium to prevent [³H]GABA from binding to GABAA sites.

Pantoyl-GABA was used as its calcium salt containing 0.5 mol of Ca²⁺ per 1 mol of drug. Thus, the total amount of Ca²⁺ was adjusted appropriately when pantoyl-GABA was added to the solution. [³H]Baclofen (51.0 Ci/mmol) and [³H]GABA (89.6 Ci/mmol) were purchased from New England Nuclear. (±)Baclofen and the calcium salt of pantoyl-GABA were gifts from Ciba-Geigy, Ltd. and Tanabe-Seiyaku Co., Ltd., respectively. Bicuculline methiodide (Pierce) and GABA (Sigma) were obtained commercially.

Pantoyl-GABA, as well as GABA, displaced [³H]GABA specifically bound to GABAA sites (Fig. 1). Pantoyl-GABA also inhibited the bindings of [³H]baclofen and [³H]GABA to GABAA sites. Its IC50 value was approximately 10⁻⁴ M in both cases (Fig. 2).

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**Fig. 1.** Displacements of [³H]GABA bound to GABAA receptors by pantoyl-GABA (●) and GABA (■). Points are means for triplicate determinations in 4 experiments.

**Fig. 2.** Displacements of [³H]baclofen (a) and [³H]GABA (b) bound to GABAA receptors by pantoyl-GABA (●), GABA (■) and baclofen (▲). Points are means for triplicate determinations in 4 experiments.
Since pantoyl-GABA is hardly metabolized in vitro as well as in vivo (13), it is probable that it acts without being metabolized to GABA.

In this study, pantoyl-GABA suppressed the bindings of ligands to both GABA_A and GABA_B sites. Pantoyl-GABA is reported to increase ACh release from rat brain via the activation of GABA_A sites (11). This effect is considered to be important for its action as an antidementia drug, because the central cholinergic function is reduced in Alzheimer’s disease (6–8). GABA_B agonist depresses release of neurotransmitters (1, 2, 14). Thus, antagonistic activity of pantoyl-GABA on GABA_B sites might cooperate to increase ACh release. The binding studies did not show whether pantoyl-GABA is an agonist or antagonist of GABA_B receptors. However, results in this study suggest that pantoyl-GABA interacts with GABA_B sites as well as GABA_A sites.

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References
1 Bowery, N.G.: Baclofen: 10 years on. Trends Pharmacol. Sci. 3, 400–403 (1982)
2 Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P., and Turnbull, M.J.: GABA receptor multiplicity. Neuropsychopharmacology 23, 219–231 (1984)
3 Olsen, R.W.: GABA-benzodiazepine-barbiturate receptor interactions. J. Neurochem. 37, 1–13 (1981)
4 Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S., Turnbull, M.J. and Warrington, R.: Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. Eur. J. Pharmacol. 71, 53–70 (1981)
5 Bowery, N.G., Hill, D.R. and Hudson, A.L.: Characteristics of GABA_B receptor binding sites on rat whole brain synaptic membranes. Br. J. Pharmacol. 78, 191–206 (1983)
6 Bartus, R.T., Dean, R.L., III, Beer, B. and Lippa, A.S.: The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408–417 (1982)
7 Coyle, J.T., Prince, D.L. and Delong, M.R.: Alzheimer’s disease: A disorder of cortical cholinergic innervation. Science 219, 1184–1190 (1983)
8 Terry, R.D. and Davies, P.: Dementia of the Alzheimer type. Annu. Rev. Neurosci. 3, 77–95 (1980)
9 Kaneda, H., Yagasaki, A., Kobayashi, T., Miyasaki, M., Hino, K., Nishimura, K., Tanino, S., Yamashita, S., Sugiyama, H., Kitajima, S., Tada, K., Hosaka, M., Takeda, M., Ozaki, S., Hariguchi, S. and Nishimura, H.: Clinical effects of Ca-Hopantenate (pantoyl-GABA) on senile and presenile organic psychotih conditions. Geriat. Med. 18, 1433–1448 (1980) (in Japanese)
10 Nakahiro, M. and Yoshida, H.: The "antidementia drug" pantoyl-y-aminobutyric acid increases high affinity uptake of choline by slices of rat brain. Neuropharmacology 25, 227–230 (1986)
11 Nakahiro, M., Fujita, N., Fukuchi, I., Saito, K., Nishimura, T. and Yoshida, H.: Pantoyl-y-aminobutyric acid facilitates cholinergic function in the central nervous system. J. Pharmacol. Exp. Ther. 232, 501–506 (1985)
12 Hill, D.R. and Bowery, N.G.: 3H-Baclofen and 3H-GABA bind to bicuculline-insensitive GABA_B sites in rat brain. Nature 290, 149–152 (1980)
13 Nishizawa, T. and Kodama, T.: Studies of homopantothenic acid. Vitamins (Japan) 33, 589–602 (1968)
14 Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, H. and Turnbull, M.: (%)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptors. Nature 283, 92–94 (1980)