Additivity and Independence of Neuroprotective Effects of $GABA_A$ and $GABA_B$ Receptor Agonists in Complete Global Cerebral Ischemia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 8, pp. 187-189, August, 2000
Original article submitted August 13, 1999

Neuroprotective effects of $GABA_B$ agonist baclofen and $GABA_A$ agonists THIP and muscimol are completely additive. $GABA_A$ and $GABA_B$ receptor antagonists block the neuroprotective effects of the corresponding agonists without cross-blocking. In fact, the blockade of combined effect of two agonists with selective $GABA_A$ and $GABA_B$ antagonists separately and in combination is not below the estimated value. Therefore, $GABA_A$ and $GABA_B$ receptor mechanisms of the neuroprotective effects independently and separately contribute to the improvement of brain resistance to global ischemia.

Key Words: *cerebral ischemia; neuroprotectors; GABA receptors; additivity of agonist effects*

Like other GABAergic substances, $GABA_A$ and $GABA_B$ receptor agonists exert pronounced neuroprotective effects (NPE) in various models of brain ischemia (BI) [1-3,7-9]. Selective antagonists of $GABA_A$ and $GABA_B$ receptors block NPE of the corresponding agonists [2]. However in order to prove the independent effects of two types of GABA receptor agonists, it is necessary to check up the possibility of cross-blocking, to study the combined effect of both agonists, and to carry out the inhibitory analysis of the effect of this combination, which was the object of the present study.

MATERIALS AND METHODS

Experiments were carried out on 404 mice (mainly CBA) of both sexes weighing 18-22 g. Highly selective $GABA_A$ and $GABA_B$ receptor agonists and antagonists were used [5,6,12]: baclofen (Sigma, RBI), bicuculline, muscimol (Serva), 2-hydroxysaclofen, THIP (RBI), and picrotoxin (gift from Dr. K. S. Raevskii). Aqueous solutions of bicuculline and picrotoxin were injected subcutaneously in a volume of 10 ml/kg, hydroxysaclofen (3 μl) into the left brain ventricle. Baclofen in a dose of 30 mg/kg was injected subcutaneously 1 h before THIP or muscimol, THIP (25 mg/kg) was injected subcutaneously, and muscimol (0.004 mg/kg) intracerebroventricularly. The animals were decapitated 1 hour after the last injection. No additional analgesia was used, because $GABA_A$ and $GABA_B$ agonists possess a pronounced antinociceptive effect [6, 12]. Decapitation model of complete global BI with evaluation of the duration of gasping was used; the results were processed using the Mann—Whitney U test. The experimental procedure was described in detail previously [4]. When studying the combination of two agonists its actual effect (E_a) was compared with the expected effect ($E_e=E_1+E_2$) and Student's t criterium was estimated by the formula $t=E_e-E_a/\overline{S}x_a$.

RESULTS

Potent NPE was characteristic of selective $GABA_B$ agonist baclofen (151% of the control level) and selective $GABA_A$ agonists THIP (161%) and muscimol (108%, Table 1). A combination of baclofen with THIP or muscimol drastically increased its effect to 294 and 234%, respectively; this did not appreciably differ

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0007-4888/00/0008-772$25.00

TABLE 1. Effects of Combination of $GABA_B$ Agonist Baclofen with $GABA_A$ Agonists THIP or Muscimol on the Duration of Gasping (in sec) in CBA Mice

Experimental series	No $GABA_A$ agonists	THIP	Muscimol
No baclofen	17.3 (14-23, n=83)	45.1* (28-65, n=21)	35.9* (29-42, n=11)
Baclofen	43.4* (32-55, n=15)	68.2*+ (50-86, n=11)	58.0*+ (37-113, n=12)

Note. $p<0.001$: *vs.* the control (no baclofen and $GABA_A$ agonists), +*vs.* the effect of $GABA_A$ agonists alone.

from the expected values for both combinations. Hence, combination of two GABA receptor agonists leads to complete summing of their individual effects ($p<0.4$ and $p<0.6$ for expected sums with THIP and muscimol, respectively).

$GABA_A$ receptors were blocked by a combination of selective antagonists bicuculline (blocks binding of $GABA_A$ agonists) and picrotoxin (blocks the chloride channel) [12]. Minor NPE of bicuculline with picrotoxin is due to presynaptic release of endogenous GABA and activation of $GABA_B$ receptors leading to analgesia [10]. In our experiments this was confirmed by the fact that selective $GABA_B$ antagonist hydroxysaclofen completely blocked $GABA_A$ antagonist NPE ($p>0.1$ *vs.* the control, n=61). Bicuculline with picrotoxin notably decreased NPE of THIP (this phenomenon was previously shown for another $GABA_A$ agonist muscimol [2]), but did not modify the effect of baclofen (Table 2). Hydroxysaclofen almost completely prevented baclofen NPE, but did not modify the activity of THIP. Therefore, each antagonist acts only through its own receptors and there are no cross-blockades.

Inhibitory analysis of NPE of $GABA_A$ and $GABA_B$ receptor agonists THIP and baclofen provided important results (Table 2). Hydroxysaclofen decreased NPE of this combination from 294 to 151%, the resultant effect being virtually the same as that of THIP alone ($p>0.1$). Bicuculline with picrotoxin decreased NPE from 294 to 140%, and the resultant effect did not differ from that of baclofen alone ($p>0.1$). This means that each selective GABA receptor blocker decreased NPE of a combination to a level corresponding to the effect of unblocked agonist. The combination of hydroxysaclofen with bicuculline and picrotoxin decreased its NPE by 2.7 times, *i. e.* to a higher degree than each antagonist separately ($p<0.05$). Actual blockade of NPE of the combination was compared with the expected value, bearing in mind that NPE of baclofen and THIP were the same. The estimated blockade under the effect of hydroxysaclofen was 43.5% (87×0.5), that under the effect of bicuculline with picrotoxin 26% (52×0.5%), and that of a combination of all antagonists 69.5%, which virtually coincided with the actual blockade (63%). On the whole, the inhibitory analysis confirmed complete additivity of NPE of $GABA_A$ and $GABA_B$ agonists used in combination.

These data indicate that $GABA_A$ and $GABA_B$ receptor-mediated mechanisms of NPE are independent and each of them contributes to the improvement of brain resistance to complete global ischemia. This is in line with the concept that biological activity of GABA, specifically the inhibition of functional activity of cerebral neurons, is realized by different independent signal-transducing systems: $GABA_A$ receptors—opening of Cl^- channel—cell polarization and $GABA_B$ receptors—$G_{O/i}$ proteins—opening of K^+ chan-

TABLE 2. Effects of GABA Antagonists on NPE of Agonists and Their Combinations

Experimental series	No antagonists	Hydroxysaclofen	Bicuculline+ picrotoxin	Combination of 3 antagonists
Baclofen				
prolongation of gasping, sec	26.1	3.4*	30.1	—
percentage of retained effect	100	13	115	—
THIP				
prolongation of gasping, sec	27.8	23.2	13.4*	—
percentage of retained effect	100	84	48	—
Baclofen+THIP				
prolongation of gasping, sec	50.9	26.7*	24.2*	18.7*
percentage of retained effect	100	52	48	37

Note. *$p<0.05$ compared to the effect of agonists alone.

nels, closing of Ca^{2+} channels, and inhibition of adenylyl cyclase [6,12]. Both independent protective mechanisms can be triggered by endogenous GABA whose extracellular concentration drastically increased under the effect of BI [9,11]. This assumption is confirmed by NPE of GABA detected by us (n=111, p<0.001) after subcutaneous (3880 μmol/kg) and, more so, intracerebroventricular (14.5 μmol/kg) injection (45 and 85-124%, respectively). Complete additivity of NPE produced by $GABA_A$ and $GABA_B$ agonists evidences the efficiency of their combinations for antiischemic protection.

The study was supported by the Russian Foundation for Basic Research (grant No. 97-04-49139).

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