1 Lukanov, J., A study on the serum factor from women with some pathologies of pregnancy and parturition, in press.
2 Lukanov, J., Milieva, E., and Tanev, A., Folia med. *24* (1982) 14.
3 Lukanov, J., Tanev, A., and Milieva, E., On the treatment of early and late toxicoses of pregnancy and spontaneous abortion, in press.
4 Lukanov, J., Tanev, A., and Sharankov, S., Akusherstvo i gynecologia *2* (1981) 91 (in Bulgarian).
5 Lukanov, J., Tanev, A., Sharankov, S., and Milieva, E., Folia med. *23* (1981) 10.
6 Mikesh, O., Laboratory Manual of Chromatographic and Similar Methods. Mir, Moscow 1982 (in Russian).
7 Northover, B.J., Br. J. Pharmac. *41* (1971) 540.
8 Osa, T., Suzuki, H., Rataze, T., and Kuriyama, H., Jap. J. Physiol. *2* (1974) 233.
9 Sharankov, S., Tanev, A. Lukanov, J., and Mincheva, T., Akusherstvo i gynecologia *3* (1981) 190 (in Bulgarian).

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Bicuculline and picrotoxin block γ-aminobutryic acid-gated Cl^- conductance by different mechanisms[1]

N. Akaike[2], K. Hattori, Y. Oomura and D.O. Carpenter

Department of Physiology, Faculty of Medicine, Kyushu University 60, Fukuoka 812 (Japan), and Center for Laboratories and Research, New York State Department of Health, Albany (New York 12201, USA), 16 February 1984

Summary. Using isolated, internally perfused bullfrog dorsal root ganglion cells we have studied the dose-response curves for γ-aminobutyric acid (GABA) in the presence of internally or externally applied GABA antagonists. With external application of antagonists the inhibition of the GABA current by bicuculline was competitive and that by picrotoxin was noncompetitive. Picrotoxin but not bicuculline blocked when internally perfused.
Key words. γ-Aminobutyric acid; bicuculline; picrotoxin; internal perfusion; competition blockade; channel blockade; frog dorsal root ganglion.

The use of internal perfusion techniques[3] on isolated neurons allows a more rigorous electrophysiologic analysis of transmitter and antagonist actions than has usually been possible. We have applied such methods to study the mechanisms of antagonism of GABA responses on bullfrog dorsal root ganglion cells by bicuculline and picrotoxin.

Materials and methods. Bullfrog lumbar dorsal root ganglion cells (primary afferent neurons) were prepared for internal perfusion as previously described[3]. To suppress the ionic currents due to ions other than Cl^- we substituted in both the internal and external perfusion solutions $TRIS^+$ for Na^+, Ca^+ for K^+, and Mg^{2+} for Ca^{2+}. The composition of these solutions is given in the table. γ-Aminobutyric acid (GABA) and/or its antagonists were added to the appropriate solutions at known concentrations. GABA was perfused at 10-min intervals. The voltage-clamp circuit used was a modification of the single-electrode clamp of Wilson and Goldner[4].

Ramp current-voltage (I–V) relations were obtained at rest, at the peak response after GABA perfusion and at the peak GABA response after perfusion of the antagonist for 10 min extracellularly or 15 min intracellularly. At low GABA concentrations all I–V curves were taken during the plateau of the response. At higher GABA concentrations the response would peak and then fall during perfusion as a result of receptor desensitization[3]. In such cases the I–V plot was obtained at the peak response as possible. Patch clamp studies were done as described by Hamill et al.[5].

Results and discussion. When bicuculline or picrotoxin was applied to the external perfusion medium each inhibited the cell's response to GABA. Relative conductance showed a sigmoidal increase with increasing GABA concentration, with a half-maximal value at 4.6×10^{-5} M (fig. 1a). After complete recovery by washing with the control solution, the preparations were pretreated with an antagonist for 10 min, followed by application of a test solution containing an antagonist and GABA. Bicuculline (10^{-5} M) produced a parallel curve shifted to the right, without altering the maximal conductance, $\bar{g}_{max}$, – a shift characteristic of competitive inhibiton. Picrotoxin depressed $\bar{g}_{max}$ but did not change the concentration of GABA producing a half-saturation response – a curve indicating noncompetitive inhibition.

I–V relations in the presence of GABA with and without bicuculline or picrotoxin are shown in figure 1b, C. In the presence of GABA alone the relation was nonlinear, presumably reflecting a decrease in the mean channel open time with hyperpolarization, as has been observed for the glutamate-mediated excitatory channels[6] and also for the GABA-activated Cl^- channels of locust muscle fibers[7]. While bicuculline caused a considerable reduction in membrane conductance, it did not alter the voltage dependence (fig. 1b). In contrast, the nonlinearity disappeared in the presence of picrotoxin (fig. 1c). Such a voltage dependence of antagonism may indicate channel blockade[8]. The difference in voltage dependence between these 2 GABA antagonists is consistent with the conclusion that they act at different sites.

If picrotoxin acts at the level of the Cl^- channel, it might be expected to block when applied internally. Figure 2A shows the currents recorded in a cell externally perfused with 10^{-5} M GABA before, during and after a 15-min internal perfusion with 10^{-5} M picrotoxin. Internal picrotoxin caused a reversible reduction of the GABA current. In contrast, internal perfusion of 10^{-5} M bicuculline had no effect on the GABA current.

Composition of perfusing solutions

	Extracellular	Intracellular
Control		
NaCl	112 mM	–
KCl	2	30 mM
$CaCl_2$	2	–
Glucose	5	–
K-aspartate	–	100
EGTA	–	0.5
Na^+, Ca^{2+}, K^+-free		
TRIS-Cl	83	–
$CsCl_2$	2	35
$MgCl_2$	5	–
TEA-Cl	23	25
4-AP	3	–
Glucose	5	–
Cs-aspartate	–	70
EGTA	–	0.5

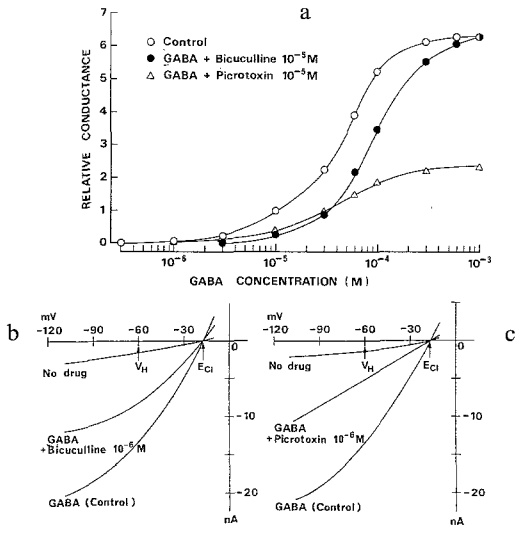

Figure 1. *a* Dose-dependent changes of relative conductance ($\bar{g}$) of primary afferent neurons in response to externally perfused test solutions: GABA alone as control (○), GABA $+10^{-5}$ M bicuculline (●) and GABA $+10^{-5}$ M picrotoxin (△). The holding potential was −70 mV; the Cl^- concentrations were 118 and 60 mM in the external and internal solutions respectively. The value of $\bar{g}$ was calculated as the ratio of 2 slope conductances estimated at the reversal potential: $\bar{g} = g_2/g_1$, where g_1 is measured under Gaba alone M) and g_2 is measured at the peak of Cl^- current evoked by GABA with or without an antagonist. Each point is the average of studies on 6 neurons. *b* and *C* I–V relationships in the absence of drugs and at a peak of current induced by 10^{-5} M GABA alone, 10^{-6} M bicuculline + GABA or 10^{-6} M picrotoxin + GABA. The curves were recorded by passing a depolarizing triangular voltage pulses (slope, 250 mV/sec) followed immediately by a mirror image hyperpolarizing pulse. The curves before and after the drugs intersect at same reversal potential, which is quite close to the value of E_{Cl} (calculated from the Nernst equation) of −17 mV. The I–V relation obtained with GABA alone is nonlinear, i.e. the conductance is voltage-dependent. Such nonlinearity is retained with bicuculline blocking *(b)* but removed by picrotoxin blocking *(c)*. This experiment was performed on 6 neurons, and thus results are typical.

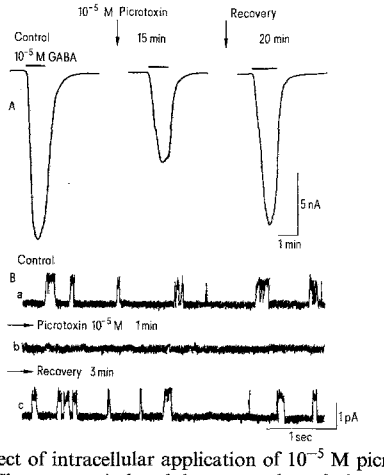

Figure 2. *A* Effect of intracellular application of 10^{-5} M picrotoxin (picrotoxin$_i$) on Cl^- current induced by external perfusion of 10^{-5} M GABA. The current was reduced about 50% within 15 min and was restored upon return to the control internal solution. The external and internal solutions contained 118 and 60 mM Cl^- respectively, at a holding potential of −70 mV. *B* Gating activity of a single Cl^- channel activated by extracellular application of 10^{-6} M GABA, at the same external and internal Cl^- concentrations as in A. The recording was stable for > 30 min after the pipette containing GABA had been sealed on the cell membrane. The inside-out patch membrane was hyperpolarized further to −53 mV from $E_{Cl} = -17$ mV. The traces are: (a) control, (b) 1 min after addition of 10^{-5} M picrotoxin to the cell interior side, and (c) 3 min after washing out of picrotoxin. These results (A and B) are typical of observations obtained from 6 neurons.

To confirm directly that picrotoxin was blocking GABA Cl^- responses at the level of the ion channel, single GABA Cl^- channel recordings were made from these neurons by using the inside-out patch clamp technique[5]. As shown in figure 2B, application of GABA (10^{-6} M) to the external surface resulted in opening of channels, which show a unitary conductance but a varied lifetime. When 10^{-5} M picrotoxin was applied to the side exposing the internal membrane surface, the channels were blocked in less than 1 min, and this blockade was rapidly reversible.

These observations indicate that bicuculline and picrotoxin block GABA responses through different mechanisms. The blockade by bicuculline is characteristic of competitive inhibition in that the dose-response curve is shifted to the right, without depression of $\bar{g}_{max}$. This implies that the binding of an antagonist molecule excludes the possibility of agonist binding. Bicuculline's action at GABA receptors has been reported to be competitive in some systems[9,10] but not in others[11], including cat dorsal root ganglion cells[12]. The reasons for these differences are not clear. The lack of effect of bicuculline on the voltage dependence of the GABA-induced current and its ineffectiveness from the cell interior in our preparation are consistent with the view that the bicuculline binding is identical to the GABA receptor or so near to it as to exclude GABA binding.

Picrotoxin blockade of GABA responses has also been reported to be competitive in some studies[13,14] but noncompetitive in others[9,11]. In our experiments, the fact that picrotoxin blocked GABA responses from either side of the membrane implies that it acts by blocking the ion channel rather than the receptor. It is interesting that this result is different from the reported for picrotoxin actions on cultured neurons[15]. GABA binding to the receptor was not reduced in the presence of picrotoxin, since the half-saturating concentration of GABA was the same in control and picrotoxin solutions (fig. 1a). The of direct blockade of single Cl^- channel currents by the internal application of 10^{-5} M picrotoxin (fig. 2B) provides further evidence for channel blockade. Thus in bullfrog dorsal root ganglion cells bicuculline binds to a site sufficiently close to the GABA receptor to be exclusionary, while picrotoxin acts on the Cl^- channels.

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2 To whom reprint requests should be addressed.
3 Hattori, K., Akaike, N., Oomura, Y., and Kuraoka, S., Am. J. Physiol. Cell Physiol. *5* (1984) C259.
4 Wilson, W.A., and Goldner, M.M., J. Neurobiol. *6* (1975) 411.
5 Hamill, O.P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F., Pflügers Arch. *391* (1981) 85.
6 Anderson, C.R., Cull-Candy, S.G., and Miledi, R., Nature *261* (1976) 151.
7 Cull-Candy, S.G., and Miledi, R., Proc. R. Soc. Lond. B *211* (1981) 527.
8 Adams, P.R., J. Membrane Biol. *58* (1981) 161.
9 Nicoll, R.A., and Wojtowicz, J.M., Brain Res. *191* (1981) 225.
10 Lebeda, F.,J., Hablitz, J.J., and Johnston, D., J. Neurophysiol. *48* (1982) 622.
11 Takeuchi, A., and Takeuchi, N., J. Physiol., Lond. *205* (1969) 377.
12 Grundfest, H., Reuben, J.P., and Rickles, W.H., J. gen. Physiol. *42* (1959) 1301.
13 Gallagher, J.P., Higashi, H., and Nishi, S., J. Physiol., Lond. *275* (1978) 263.
14 Shank, R.P., Pong, S.F., Freeman, A.R., and Graham, L.T. Jr, Brain Res. *72* (1974) 71.
15 Barker, J.L., McBurney, R.N., Mathers, D.A., and Vaughn, W., J. Physiol., Lond. *308* (1980) 18P.

0014-4754/85/010070-02$1.50 + 0.20/0