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Effects of benzodiazepines and non-benzodiazepine compounds on the GABA-induced response in frog isolated sensory neurones

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1 The effects of benzodiazepines and non-benzodiazepine compounds on the γ-aminobutyric acid (GABA)-induced chloride current (I_{Cl}) were studied in frog isolated sensory neurones by use of a concentration-jump (termed 'concentration-clamp') technique, under single-electrode voltage-clamp conditions. The drugs used were classified into four categories as follows: full benzodiazepine receptor agonists (diazepam, clonazepam, nitrazepam, midazolam, clotiazepam and etizolam), partial agonists (CL 218,872, Ro 16-6028, Ro 17-1812 and Ro 23-0364), inverse agonists (Ro 15-3505, FG 7142 and β-CCE) and a benzodiazepine receptor antagonist, Ro 15-1788 (flumazenil).

2 All full agonists at concentrations of 3×10^{-6} M or less increased dose-dependently the peak amplitude of I_{Cl} elicited by 3×10^{-6} M GABA to twice to three times larger than the control. However, no further augmentation of the GABA response was observed at concentrations of 1×10^{-5} M or higher. Partial agonists also showed a dose-dependent augmentation of the GABA response at concentrations ranging from 3×10^{-8} M to 3×10^{-5} M, but their efficacies of augmentation of the GABA response were only about half or less of those of full agonists. Of the inverse agonists, β-CCE had a unique dose-dependent effect on the GABA response. β-CCE reduced dose-dependently the GABA response at concentrations of less than 3×10^{-6} M, but augmented it at concentrations of 3×10^{-5} M and 6×10^{-5} M. The inverse agonists reduced dose-dependently the GABA response. The benzodiazepine antagonist, flumazenil, slightly augmented the GABA response at concentrations between 3×10^{-7} M and 3×10^{-5} M.

3 These results show clear differences in the effects on the GABA response between these four categories of compounds known to affect the benzodiazepine recognition site of the GABA/benzodiazepine receptor-chloride channel complex. Our experimental system of frog isolated sensory neurones and a 'concentration-clamp' technique appears to be useful for evaluating efficacy of compounds on responses mediated by the GABA/benzodiazepine receptor-chloride channel complex.

Introduction

Anxiety is a ubiquitous symptom associated with medical as well as psychiatric disorders, some of which can be diagnosed and effectively treated. Currently, the most useful drugs seem to be the benzodiazepine derivatives which facilitated γ-aminobutyric acid (GABA) responses via the benzodiazepine receptor at the GABA/benzodiazepine receptor-ionophore complex (Costa *et al.*, 1975; Haefely *et al.*, 1975; Möhler & Okada, 1977). However, as the dose of benzodiazepines is increased, the anxiolytic effect progresses to sedation, sedation to sleep and/or anticonvulsive activity (Harvey, 1985; Petersen *et al.*, 1986). In the treatment of anxiety, other pharmacological actions of the drugs become undesirable side effects. Therefore, compounds that possess an anxioselective anxiolytic effect (pure anxiolytic action) are desirable. Recently, non-benzodiazepine compounds have been studied, for example cyclopyrolones, triazolopyridazines, pyrazoloquinolines and quinoline derivatives (Blachard *et al.*, 1979; Lippa & Crichett, 1979; Williams, 1983; Blanchard & Jolon, 1983). Many of them lack sedative, hypnotic and/or anticonvulsive activity. However, the compounds which affect the benzodiazepine receptor at the GABA/benzodiazepine receptor-ionophore complex can be

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Table 1 Compositions of internal and external test solutions (in mM)

Internal solution	CsCl 95	Cs-aspartate 10	TEA-Cl 25	HEPES 10	EGTA-Ca^{2+} 1×10^{-8} M	buffer Ca^{2+}
External solution	Tris-Cl 89	CsCl 2	$MgCl_2$ 5	TEA-Cl 25	Glucose 5	HEPES 10

The pH of internal and external solutions was adjusted to 7.2 and 7.4, respectively, with appropriate Tris-base.

classified into at least three categories: benzodiazepine receptor agonists (full and partial), inverse agonists and antagonists (Richards & Möhler, 1984). Such a classification has been based mainly on their pharmacological profile after their affinities to the benzodiazepine receptor had been established biochemically (Polc *et al.*, 1982; Möhler & Richards, 1983; Richards & Möhler, 1984); there have been relatively few systematic comparisons made with electrophysiological techniques (Chan & Farb, 1985; Sigel & Baur, 1988).

For the last five years we have carried out physiological and pharmacological studies on the $GABA_A$ response with single neurones isolated from frog dorsal root ganglia and a 'concentration-clamp' technique. Evidence has accumulated which suggests that this experimental system is useful for evaluating activity at the GABA receptor-ionophore complex (Hattori *et al.*, 1984; 1986; Akaike *et al.*, 1985a,b; 1986; 1987a,b,c; Inoue *et al.*, 1986; Akaike, 1989). In the present study, we have compared the effects of several benzodiazepines and non-benzodiazepine compounds on the $GABA_A$ receptor response to see if our experimental system adequately distinguishes the different compounds originally classified in biochemical studies. Part of this study has been presented in a preliminary form in Japanese (Akaike *et al.*, 1987d).

Methods

The experimental methods used were as described previously (Hattori *et al.*, 1984; Ishizuka *et al.*, 1984; Akaike *et al.*, 1986). In brief, dorsal root ganglia isolated from bull-frog (*Rana catesbiana*) were digested in normal Ringer solution containing 0.3% collagenase and 0.05% trypsin at pH 7.4 for 15 to 20 min at 37°C. During the enzyme treatment, the preparation was gently agitated by bubbling 95% O_2 and 5% CO_2. Thereafter, single neurones were mechanically isolated from the digested ganglia with fine pins under a binocular microscope. Isolated neurones were maintained in a solution which contained equal parts of Eagle's minimum essential medium (Nissui, Tokyo, Japan) and normal Ringer solution at a room temperature of 22 to 25°C. Isolated neuronal cell bodies were perfused internally and externally with respective test solutions (Table 1) for recording the chloride current (I_{Cl}), by a suction pipette technique (Ishizuka *et al.*, 1984). Neurones were voltage-clamped at a holding membrane potential of −50 mV with a single-electrode voltage-clamp system (Ishizuka *et al.*, 1984). Drugs were applied by use of a 'concentration-clamp' technique (see Figure 1 of Akaike *et al.*, 1986).

Clonazepam, nitrazepam, midazolam, flumazenil, Ro 15-3505 (ethyl-7-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate), Ro 16-6028 (tert-butyl(s)-8-bromo-11,12,13,13a-tetra-hydro-9-oxo-9H-imidazo[1,5-a]-pyrrolo-[2,1-c][1,4]benzodiazepine-1-carboxylate), Ro 17-1812 (cyclopropylmethyl(s)-8-chloro-12,12a-dihydro-9-oxo-9H,11H-aceto-[2,1-c]-imidazo-[1,5-a]-[1,4]benzodiazepine-1-carboxylate) and Ro 23-0364

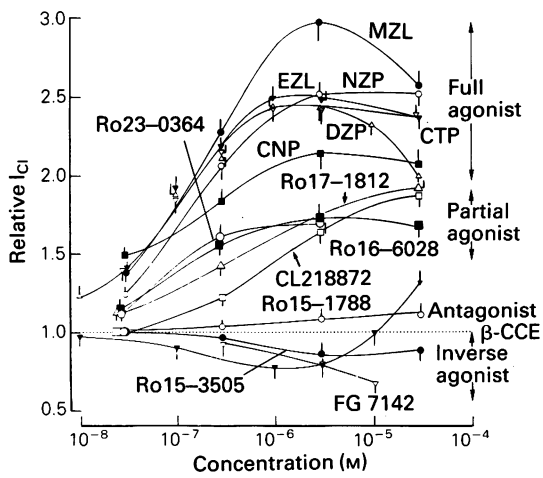

Figure 1 Concentration-response relationships for the facilitatory and inhibitory actions of the benzodiazepines and non-benzodiazepine compounds on the response elicited by 3×10^{-6} M γ-aminobutyric acid (GABA). Each point shows the mean value of four to six experiments; vertical lines show s.e.mean. The dotted line indicates the current amplitude induced by 3×10^{-6} M GABA alone (the control level). All values are normalized to the control level. MZL (midazolam), NZP (nitrazepam), CNP (clonazepam), CTP (clotiazepam), DZP (diazepam) and EZL (etizolam). Holding potential was −50 mV.

(6-(2-chlorophenyl)-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxamide) were provided by Dr Scherschlicht (F. Hoffman – La Roche & Co., Ltd., Basel, Switzerland). Etizolam, clotiazem, CL 218872 (3-methyl-6-(3-trifluoromethyl-phenyl)-1,2,4-triazolo[4,3-b]pyridazine), FG 7142 (N-methyl-β-carboline-3-carboxamide) and β-CCE (ethyl-β-carboline-3-carboxylate) were synthesized and provided by Yoshitomi Pharmaceutical Industries (Yoshitomi, Japan). Diazepam was purchased from Sigma (St. Louis, U.S.A.). All test drugs were initially dissolved in dimethyl sulphoxide (DMSO, Sigma) and diluted with the external test solution just before use. DMSO at final concentrations (0.2% or less) did not affect the GABA response. Only GABA, of the drugs tested, directly elicited the I_{Cl} at the concentrations used in this study. The different agents (the benzodiazepines and non-benzodiazepine compounds) were simultaneously applied to the neurones with 3×10^{-6} M GABA; at this concentration GABA produces very slow desensitization (Akaike *et al.*, 1985a; Hattori *et al.*, 1986). All values of the current amplitude were normalized, relative to the current amplitude produced by 3×10^{-6} GABA. Control current amplitude of the GABA response was 0.437 ± 0.009 nA (mean ± s.e., $n = 68$).

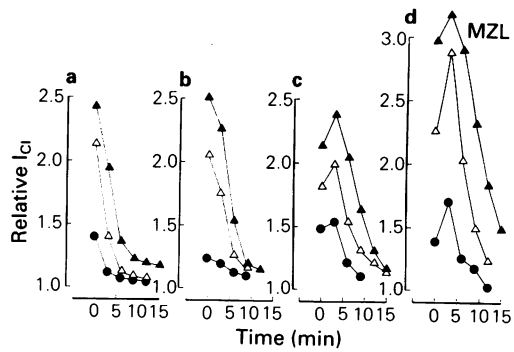

Figure 2 Time course of the recovery from the drug-induced augmentation of the response to γ-aminobutyric acid (GABA) (3×10^{-6} M) after washout of (a) diazepam, (b) nitrazepam, (c) clonazepan and (d) midazolam. The compounds were used at 3 different concentrations: (●) 3×10^{-8} M, (△) 3×10^{-7} M and (▲) 3×10^{-6} M. Abscissae indicate the time after washout of the agent. Zero point shows the GABA response just before washout of the agent. Ordinate scales: the current amplitude of the GABA response relative to that elicted by 3×10^{-6} M GABA alone. The results are typical of observations obtained from four experiments.

Results

As shown in Figure 1, the simultaneous application of the full benzodiazepine receptor agonists (diazepam, clonazepam, nitrazepam, midazolam, etizolam and clotiazepam) at concentrations between 3×10^{-7} M and 3×10^{-6} M potentiated the GABA response to two to three times the control level induced by GABA alone. Increasing the concentration of these agents beyond 3×10^{-6} M did not produce further augmentation of the GABA response. The dose-response relationships for both midazolam and diazepam were bell-shaped. Recovery from the augmentation, induced by the concentrations used in Figure 1, was complete within 30 min after washing out the agent. However, the time course of recovery from the augmentation of the GABA response induced by midazolam or clonazepam was different from those of the other full agonists. After wash out of midazolam or clonazepam, a further increase of the GABA response was observed ('after-wash' augmentation), while with diazepam and nitrazepam the response returned smoothly toward the control level after wash out (Figure 2). The partial agonists (CL 218,872, Ro 16-6028, Ro 17-1812 and Ro 23-0364) produced dose-dependent increases in the GABA response at a greater range of concentrations (3×10^{-8} M to 3×10^{-5} M), but their effects on the GABA response were only about half or less than those induced by the full agonists. Recovery was also complete within 30 min of washout. The benzodiazepine bases are generally lipophilic and might well penetrate into the membrane bilayer. Trapping in the membrane could be the reason for a slow recovery and/or 'after-wash' augmentation. Removal of an overoptimal concentration of a benzodiazepine in the extracellular medium by the first wash could well liberate enough trapped benzodiazepine to yield a somewhat higher response to GABA.

Data from ligand binding experiments suggest that Ro 15-1788 is a benzodiazepine antagonist (for a review, Richards & Möhler, 1984), but in our experiment it slightly increased the GABA response, indicating that flumazenil is a very weak partial agonist rather than a 'pure' benzodiazepine antagonist. Recovery was complete within 30 min after washout of this agent. Two inverse agonists (Ro 15-3505 and FG 7142) dose-dependently reduced the GABA-induced current amplitude at concentrations of 3×10^{-7} M or more. However β-CCE produced a unique dose-dependent effect on the GABA response (Figure 1). At concentrations of 1×10^{-8} M to 1×10^{-6} M it dose-dependently reduced the GABA response (Figure 3a). However, the inhibitory action of β-CCE on the GABA response was reduced at concentrations between 3×10^{-6} M and 1×10^{-5} M and it potentiated the GABA response at concentrations of 3×10^{-5} M and 6×10^{-5} M (Figure 3b,c).

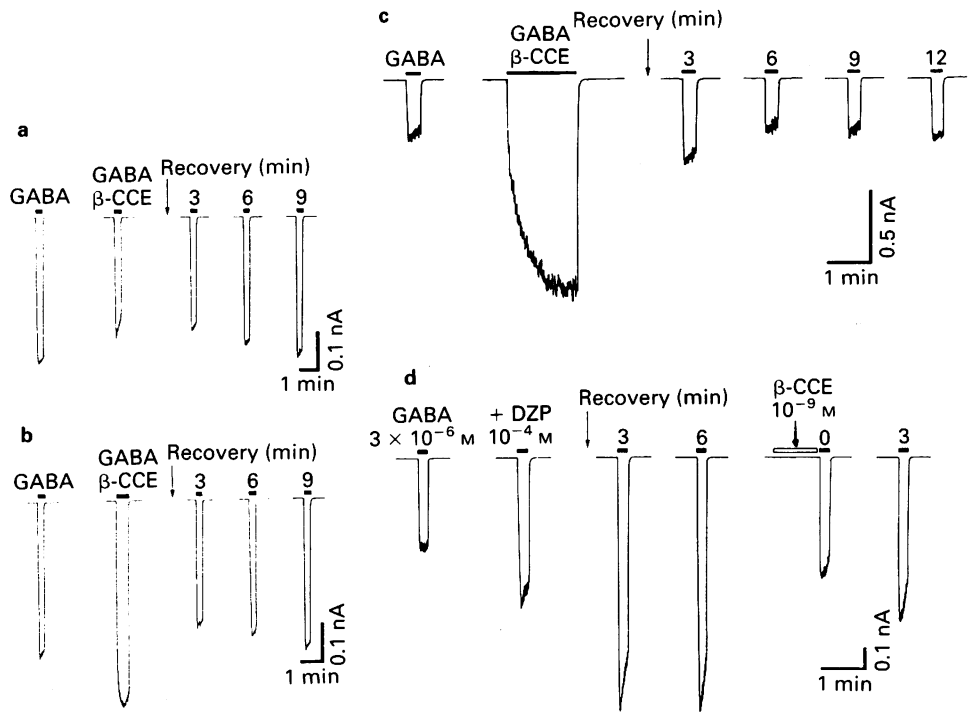

Figure 3 Inhibitory and facilitatory actions of β-CCE on the response elicited by 3×10^{-6} M γ-aminobutyric acid (GABA). Concentrations of β-CCE were 1×10^{-6} M (a), 3×10^{-5} M (b), 6×10^{-5} M (c) and 1×10^{-9} M (d). β-CCE was simultaneously applied to the neurone with 3×10^{-6} M GABA in each case. In (d), 1×10^{-4} M diazepam (DZP) was simultaneously applied with 3×10^{-6} M GABA. To see the antagonistic action of β-CCE on the GABA response modulated by diazepam the neurone was treated with 1×10^{-9} M β-CCE for 1 min before applying 3×10^{-6} M GABA alone. Holding potential was −50 mV.

Recovery from the effect of this inverse agonist was complete within 20 min of washout. However, the time course of recovery from the effect of β-CCE varied with different concentrations. As shown in Figure 3a, the current amplitude of the GABA response time-dependently recovered toward the control level at a concentration of 1×10^{-6} M. A similar change in the current amplitude after washout was observed at a β-CCE concentration of 3×10^{-5} M, although at this concentration β-CCE increased with GABA response (Figure 3b). With 6×10^{-5} M β-CCE, the GABA response was still larger than the control shortly after washing, it then recovered with a similar time course to that seen with 1×10^{-6} M and 3×10^{-5} M β-CCE, i.e. the current amplitude of the GABA response became smaller than the control and then returned to the control level. Another interesting effect of β-CCE was observed in the experiment shown in Figure 3d. Diazepam at 1×10^{-4} M slightly augmented the GABA response, as expected from its 'bell-shaped' dose-response curve. When a high concentration of diazepam was used, it augmented the GABA response after washout, as seen with midazolam and clonazepam. Such potentiation by 1×10^{-4} M diazepam persisted for about 20 min after washing. This effect was greatly reduced by a low concentration (1×10^{-9} M) of β-CCE, which did not affect the GABA response directly.

Discussion

As illustrated in Figure 1, our experimental system using a 'concentration-clamp' technique and frog isolated single neurones could clearly distinguish effects of the different categories of drugs, known to act on the benzodiazepine receptor and defined by pharmacological and biochemical studies (Polc *et al.*, 1982; for a review, Richards & Möhler, 1984). Of the full agonists examined, midazolam and diazepam produced a 'bell-shaped' dose-dependent effect on the

GABA response at concentrations ranging from 1×10^{-8} M to 3×10^{-5} M (Figure 1). The reason for the 'bell-shaped' dose-response curve is unknown, but high concentrations of these benzodiazepines may directly block GABA-gated chloride channels or may non-specificially perturb membrane functions. Although they have similar dose-dependent effects, there are interesting differences between them. With midazolam, the 'after-wash' augmentation of the GABA response was observed at all the concentrations used (3×10^{-8} M or more), whereas this was only observed with diazepam at a high concentration (1×10^{-4} M) (Figure 2). Yet both agents equally augmented the GABA response in the concentration range 3×10^{-8} M to 3×10^{-5} M. Therefore, it is possible that some full agonists have two actions on the GABA/benzodiazepine receptor-ionophore complex with a different dose-dependence: one is to increase the affinity of the GABA/benzodiazepine receptor to GABA and the other may be to hinder directly some functions of the GABA receptor-ionophore complex or the GABA-GABA receptor interaction, not yet specified. As the dose of the benzodiazepines is increased, the anxiolytic effect progresses to sedative, sedative to hypnosis and/or anticonvulsive effects. Therefore, partial agonists may be ideal anxioselective anxiolytics, since their potencies in augmenting the GABA response are relatively weak and dose-dependent changes in their actions on the GABA response are moderate (Figure 1). As shown in Figure 1, the degree of potentiation of the GABA response induced by partial agonists (CL 218,872, Ro 16-6028, Ro 17-1812 and Ro 23-0364) at the maximum concentration (3×10^{-5} M) seems to be obtained at concentrations around 1×10^{-7} M when full agonists are used. Clonazepam (CNP) has been regarded for many years as a full agonist and has been used as an antiepileptic. Recently, it has been shown, in a preliminary study, that CNP in a certain dose range antagonizes the effect of diazepam in a behavioural test (Bonetti *et al.*, 1987). As shown in Figure 1, the maximum efficacy of CNP is located between those of full agonists and partial agonists. Therefore CNP may be a partial agonist with a higher intrinsic efficacy than most other partial agonists.

Flumazenil has been shown to bind with high affinity to the benzodiazepine receptor site and to antagonize a variety of the actions induced by the benzodiazepines (Hunkeler *et al.*, 1981). However, two opposite, excitatory and inhibitory, actions on the GABA response have been obtained in electrophysiological studies (Chan & Farb, 1985; Sigel & Baur, 1988). Thus, at the concentration range 3×10^{-8} M to 1×10^{-6} M, flumazenil augmented the GABA response of cultured neurones isolated from embryonic chick spinal cord (Chan & Farb, 1985), while it reduced the response mediated by the chick forebrain GABA receptor expressed in *Xenopus* oocytes (Sigel & Baur, 1988). At present there is no explanation for this difference. In our case flumazenil slightly, but dose-dependently, increased the GABA response at concentrations similar to those previously used (Chan & Farb, 1985; Sigel & Baur, 1988). In spite of the wide use of the term 'benzodiazepine antagonist', flumazenil seems to possess a small intrinsic action and it may thus be termed a partial antagonist in our case. Of the inverse agonists examined here, Ro 15-3505 and FG 7142 reduced the GABA response as expected. However, β-CCE produced complicated actions on the GABA response, as observed previously (Akaike *et al.*, 1987d; Sigel & Baur, 1988). These results suggest that β-CCE may be an inverse agonist possessing 'intrinsic activity' on the benzodiazepine receptor. However, the inverse agonistic activity of the agent could be observed at a concentration of 1×10^{-9} M when the GABA receptor-ionophore complex was previously modulated by a full agonist (Figure 3d). Therefore, the specificity of β-CCE as an inverse agonist is thought to be high.

In conclusion, our experimental system of frog isolated sensory neurones and a 'concentration-clamp' technique appears to be a useful one for evaluating the efficacy of benzodiazepines and non-benzodiazepine compounds on the responses mediated by the GABA receptor-ionophore complex.

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References

AKAIKE, N. (1989). GABA-gated Cl^- currents and their regulation by intracellular free Ca^{2+}. In *Chloride Channels and Carries in Nerve Muscle and Glial Cells*. ed. Alvarez-Leebmans, F.J. & Russel, J. New York: Plenum Press, (in press).

AKAIKE, N., HATTORI, K., INOMATA, N. & OOMURA, Y. (1985a). γ-Aminobutyric acid and pentobarbitone-gated chloride currents in internally-perfused frog sensory neurones. *J. Physiol.*, **360**, 367–386.

AKAIKE, N., HATTORI, K., OOMURA, Y. & CARPENTER, D.O. (1985b). Bicuculin and picrotoxin block γ-aminobutyric-acid-gated Cl^{-1} conductance by different mechanisms. *Experientia*, **41**, 70–71.

AKAIKE, N., INOUE, M. & KRISHTAL, O.A. (1986).

'Concentration-clamp' study of γ-aminobutyric-acid-induced chloride current kinetics in frog sensory neurones. *J. Physiol.*, **379**, 171–185.

AKAIKE, N., INOMATA, N. & TOKUTOMI, N. (1987a). Contribution of chloride shifts to the fade of γ-aminobutyric-acid-gated currents in internally perfused frog sensory neurones. *J. Physiol.*, **391**, 219–234.

AKAIKE, N., MARUYAMA, T., SIKDAR, S.K. & YASUI, S. (1987b). Sodium-dependent suppression of γ-aminobutyric-acid-gated chloride currents in frog sensory neurones. *J. Physiol.*, **392**, 543–562.

AKAIKE, N., MARUYAMA, T. & TOKUTOMI, N. (1987c). Kinetic properties of the pentobarbitone-gated chloride current in frog sensory neurones. *J. Physiol.*, **394**, 85–98.

AKAIKE, N., YAKUSHIJI, T., TOKUTOMI, N., HATTORI, K. & OOMURA, Y. (1987d). (Translated into English) Neurophysiological mode of action of anxiolytics. In *Biowarning System in the Brain (Cont.)* ed. Takagi, H., Oomura, Y. & Ito, M. pp. 253–267. University of Tokyo Press: Tokyo (in Japanese).

BLANCHARD, J.C., BOIREAU, A., GRARRET, C. & JOLOU, L. (1979). *In vitro* and *in vivo* inhibition by zopiclone of benzodiazepine binding to rodent brain receptor. *Life Sci.*, **24**, 2417–2420.

BLANCHARD, J.C. & JOLOU, L. (1983). Suriclone. *J. Neurochem.*, **40**, 601–607.

BONETTI, E.P., POLC, P., LAURENT, J.P., SCHAFFNER, R., SCHOCH, P. & HAEFELY, W. (1987). Clonazepam is a partial agonist at the benzodiazepine receptor. *Neuroscience*, **2**, (suppl), 245p.

CHAN, C.Y. & FARB, D.H. (1985). Modulation of neurotransmitter action: control of the γ-aminobutyric acid response through the benzodiazepine receptor. *J. Neurosci.*, **5**, 2365–2373.

COSTA, E., GUIDOTTI, A. & MAO, C.C. (1975). Evidence for the involvement of GABA in the action of benzodiazepines: studies on rat cerebellum. In *Mechanisms of Action of Benzodiazepines.* ed. Costa, E. & Greengard, P. pp. 113–130. New York: Raven Press.

HAEFELY, W., KULCSAR, A., MÖHLER, H., PIERI, L., POLC, P. & SCHAFFNER, R. (1975). Possible involvement of GABA in the central actions of benzodiazepines. In *Mechanisms of Action of Benzodiazepines.* ed. Costa, E. & Greengard, P. pp. 131–151. New York: Raven Press.

HARVEY, S.C. (1985). Hypnotics and sedatives. In *The Pharmacological Basis of Therapeutics.* ed. Gilman, A.G., Goodman, L.S., Rall, T.W. & Murad, F. pp. 339–371. New York: Raven Press.

HATTORI, K., AKAIKE, N., OOMURA, Y. & KURAOKA, S. (1984). Internal perfusion studies demonstrating GABA-induced chloride responses in frog primary afferent neurons. *Am. J. Physiol.*, **246**, C259–265.

HATTORI, K., OOMURA, Y. & AKAIKE, N. (1986). Diazepam action on γ-aminobutyric-acid-activated chloride currents in internally-perfused frog sensory neurons. *Cell. Molec. Neurobiol.*, **6**, 307–323.

HUNKELER, W., MÖHLER, H., PIERI, L., POLC, P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. & HAEFELY, W. (1981). Selective antagonists of benzodiazepines. *Nature*, **290**, 514–516.

INOUE, M., OOMURA, Y., YAKUSHIJI, T. & AKAIKE, N. (1986). Intracellular calcium ions decrease the affinity of the GABA receptor. *Nature*, **324**, 156–158.

ISHIZUKA, S., HATTORI, K. & AKAIKE, N. (1984). Separation of ionic currents in the somatic membrane of frog sensory neurons. *J. Memb. Biol.*, **78**, 19–28.

LIPPA, A.S. & CRICHETT, D.J. (1979). Benzodiazepine receptors. *Pharmacol. Biochem. Behav.*, **10**, 831–843.

MÖHLER, H. & OKADA, T. (1977). Benzodiazepine receptors: demonstration in the central nervous system. *Science*, **198**, 849–851.

MÖHLER, H. & RICHARDS, J.G. (1983). Receptors for anxiolytic drugs. In *Anxiolytics: Neurochemical, Behavioural and Clinical Perspectives.* ed. Malick, J.B., Ennas, J. & Yamamura, H.I., pp. 15–40. New York: Raven Press.

PETERSEN, E.N., JENSEN, L.H., DREJER, J. & HONORE, T. (1986). New perspectives in benzodiazepine receptor pharmacology. *Pharmacopsychiat.*, **19**, 4–6.

RICHARDS, J.G. & MÖHLER, H. (1984). Benzodiazepine receptors. *Neuropharmacology*, **23**, 233–242.

POLC, P., BONETTI, E.P., SCHAFFNER, R. & HAEFELY, W. (1982). A 3 states model of the benzodiazepine receptor explains the interactions between the benzodiazepine antagonist Ro 15-1788, benzodiazepine tranquilizers, beta-carbolines and phenobarbitone. *Arch. Pharmacol*, **321**, 260–264.

SIGEL, E. & BAUR, R. (1988). Allosteric modulation by benzodiazepine receptor ligands of $GABA_A$ receptor-channel expressed in *Xenopus* oocytes. *J. Neurosci.*, **8**, 289–295.

WILLIAMS, M. (1983). Anxioselective anxiolytics. *J. Med. Chem.*, **26**, 619–628.

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