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PHOSPHOLIPIDS AND BENZODIAZEPINE RECOGNITION SITES OF BRAIN SYNAPTIC MEMBRANES

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Summary—Benzodiazepine binding to cerebral synaptic membranes and its modulation by membraneous phospholipids were studied. [^{3}H]diazepam and [^{3}H]flunitrazepam binding to the cerebral synaptic membrane were saturable and consisted of one component with a high affinity. Both [^{3}H]diazepam and [^{3}H]flunitrazepam binding was significantly decreased by pretreatment of the membrane with trypsin and pronase. In contrast, treatment of the membrane with phospholipase C and phospholipase A_2, induced a significant increase in binding. Kinetic studies have indicated that the treatment with phospholipase C induces an increase in density of the receptors, whereas treatment with phospholipase A_2 increases the affinity of the receptor. The addition of phospholipids, such as phosphatidyl serine and phosphatidic acid, abolished the increase in [^{3}H]diazepam binding induced by phospholipase C and phospholipase A_2 treatment. The GABA-induced increase in [^{3}H]diazepam binding was significantly attenuated following phospholipase A_2 treatment, but not in the control or in phospholipase C-treated membranes. In addition, the binding of [^{3}H]flunitrazepam to the solubilized benzodiazepine receptor from the synaptic membrane was not affected by phospholipase C and phospholipase A_2 treatments.

The present results indicate that the benzodiazepine receptor binding in synaptic membranes is modulated, at least in part, by membraneous phospholipids susceptible to treatment with phospholipase C and phospholipase A_2.

It has been well documented that a high affinity binding site for benzodiazepines is present in the brain (Braestrup and Squires, 1977; Braestrup, Albrechtsen and Squires, 1977; Möhler and Okada, 1977a, b; Speth, Wastek, Johnson and Yamamura, 1978; Squires and Braestrup, 1977; Williamson, Paul and Skolnick, 1978a, b). This site appears to be a pharmacologically relevant receptor for benzodiazepines, since a good correlation is present between the magnitude of the affinity of various benzodiazepines for the binding site and clinical potencies (Randall, Schallek, Sternbach and Ning, 1974). Although it is still unknown whether or not an endogenous ligand for the receptor exists, studies on the receptor seem to be important for elucidating the central action of benzodiazepines. On the other hand, various biochemical (Brilely and Langer, 1978; Costa, Guidotti, Mao and Suria, 1975; Haefely, Kulcsar, Möhler, Pieli, Polc and Schaffner, 1975; Karobath and Sperk, 1979; Martin and Candy, 1978; Tallmann, Thomas and Gallager, 1978) and electrophysiological (Gallager, 1978) studies have indicated that the action of benzodiazepines is closely related to that of γ-aminobutyric acid (GABA). Furthermore, it has been suggested that the function of the GABA receptor may be regulated by an endogenous inhibitor (Guidotti, Toffano and Costa, 1978; Toffano, Guidotti and Costa, 1978; Yoneda and Kuriyama, 1980), and benzodiazepines may exert their action via the inactivation of this inhibitor on GABA receptor binding (Guidotti *et al.*, 1978; Toffano *et al.*, 1978).

In the present study, an attempt has been made to examine whether or not a similar type of endogenous modulating mechanism for the benzodiazepine receptor is present in the brain.

Key words: benzodiazepine receptor, membraneous phospholipids, solubilization, GABA stimulation.

METHODS

Preparation of synaptic membranes and solubilized fractions

Male Wistar rats weighing 180–200 g were decapitated and the brain was removed. The crude synaptic membrane was prepared according to the method of Enna and Snyder (1976). The prepared membrane was washed three times with 50 mM Tris–HCl buffer (pH 7.4) and stored at −20°C for at least 12 hr. The frozen membrane was thawed, resuspended in Tris-HCl buffer and centrifuged at 48,000 *g* for 20 min. The resultant pellet was resuspended with Tris–HCl buffer and used for the binding assay.

The solubilized fraction from cerebral synaptic membranes was obtained by treating the synaptic membrane fraction with 50 mM Tris–citrate buffer (pH 7.1) containing 1% Nonidet P-40 followed by centrifugation at 100,000 *g* for 60 min. The supernatant thus obtained was dialyzed against Tris–citrate buffer containing 0.1% Nonidet P-40 for 2 hr at 2°C, and subjected to ammonium sulfate precipitation (40% saturation). The resultant precipitate was resuspended with Tris–citrate buffer containing 0.1% Nonidet P-40 and centrifuged at 100,000 *g* for 30 min. The super-

natant was redialyzed against Tris–citrate buffer containing 0.1% Nonidet P-40 at 2°C for 12 hr and this fraction was subjected to the binding assay.

Assay of benzodiazepine binding

For the measurement of benzodiazepine binding to synaptic membranes, membraneous preparations (containing 0.2–0.3 mg protein) were incubated with 0.5 nM [^{3}H]diazepam or [^{3}H]flunitrazepam at 4°C for 20 or 60 min, respectively. In the case of the solubilized fraction, the same experimental procedure was employed as for the membrane fractions except that polyethyleneglycol was added to the reaction mixture in a final concentration of 15% (w/v). Each incubation was terminated by filtrating under vacuum through a Whatmann GF/B filter and then the filter was rinsed twice with 3 ml of ice-cold Tris–HCl buffer. Radioactivity trapped on the filter was measured by liquid scintillation spectrometry, using a toluene-Triton X-100 scintillation cocktail. The specific binding was calculated by subtracting the amount of nonspecific binding, found in the presence of 10^{-5} M diazepam (for [^{3}H]diazepam binding) or clonazepam (for [^{3}H]flunitrazepam binding), from the total binding. The GABA-induced stimulation of benzodiazepine binding was determined in the presence of 10^{-5} M GABA.

Procedure for treatment with various enzymes and drugs and of the subsequent addition of various phospholipids

Treatments with various enzymes were performed by preincubating the membrane and solubilized fractions at 37°C for 30 min before subjecting them to the binding assay. In the case of treatments with phospholipase C, phospholipase A_2 and phospholipase D, 1 mM $CaCl_2$ was added to the preincubation medium. On the other hand, treatment with digitonin, nystatin and polymixin B was carried out by incubating the membrane at 37°C for 30 min. For examining the effect of the addition of various phospholipids, each phospholipid was homogenized by a Polytron homogenizer for 30 sec with membrane fractions. After preincubating at 37°C for 30 min, 0.1 ml aliquot of the resultant suspension (containing 500 μg/ml of each phospholipid) was used for the binding assay.

Measurement of protein

Protein content was measured by the method of Lowry, Rosebrough, Farr and Randall (1951).

Various chemicals used

[*N*-methyl-^{3}H]diazepam (76.8 Ci/mmol) and [*N*-methyl-^{3}H]flunitrazepam (75 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Diazepam and clonazepam were kindly provided from the Takeda Chemical Co. Ltd, Osaka and Sankyo Co. Ltd, Tokyo, respectively. The following compounds were also purchased from the Sigma Chemical Co., St Louis. MO.: phospholipase C, phospholipase A_2, phospholipase D, trypsin, pronase, neuraminidase, β-galactosidase, lipoxygenase, phosphatidic acid, L-α-phosphatidyl serine, L-α-phosphatidyl choline, L-α-phosphatidyl ethanolamine, glycerophosphoethanolamine, *O*-phosphoethanolamine, digitonin, nystatin and polymixin B.

RESULTS

Kinetic analysis of [^{3}H]diazepam and [^{3}H]flunitrazepam binding to cerebral synaptic membranes

The synaptic preparation was incubated with an increasing concentration (0.5–40.5 nM) of [^{3}H]diazepam and [^{3}H]flunitrazepam to examine the saturability of the specific binding site. The Scatchard analysis (Fig. 1) provided apparent dissociation constants (K_D) of 6.36 ± 0.75 nM ([^{3}H]diazepam) and 1.50 ± 0.08 nM ([^{3}H]flunitrazepam), and a maximal number of the binding sites (B_{max}) of 0.94 ± 0.11 pmol/mg protein ([^{3}H]diazepam) and 1.12 ± 0.06 pmol/mg protein ([^{3}H]flunitrazepam), respectively. These results indicate that the affinity of flunitrazepam for the benzodiazepine receptor was higher than that of diazepam as reported previously by several investigators (Braestrup *et al.*, 1977; Möhler and Okada, 1977a, 1978; Speth *et al.*, 1978a; Squires and Braestrup, 1977). Moreover, both [^{3}H]diazepam and [^{3}H]flunitrazepam binding was significantly enhanced by the addition of 10^{-5} M GABA (see Fig. 1), and this enhancement was antagonized by 10^{-4} M bicuculline, a specific antagonist for the GABA receptor. These data support the assumption, proposed by various laboratories, that GABA-induced stimulation of benzodiazepine receptor binding may be mediated through a GABA receptor. In fact, the Scatchard analysis showed that GABA increased the affinity of the benzodiazepine receptor site without altering the B_{max} value as reported previously.

Effect of various enzymatic treatments on the benzodiazepine receptor

To examine some characteristics of the receptor site, synaptic membranes were pretreated with various enzymes at 37°C for 30 min before being subjected to the binding assay. The [^{3}H]diazepam binding was significantly reduced by pretreatment of the membranes with proteolytic enzymes, such as trypsin and pronase (Table 1), while pretreatment with neuraminidase, β-galactosidase, lipoxygenase and phospholipase D had no significant effect on the binding. These differential effects of enzymatic treatments were also detected when the amount of each enzyme was increased up to 10-fold as compared with that described in Table 1.

On the other hand, treatment with phospholipase C and phospholipase A_2 significantly increased [^{3}H]diazepam binding, and this phenomenon was also seen in the binding study using [^{3}H]flunitrazepam as a radioligand (Table 2).

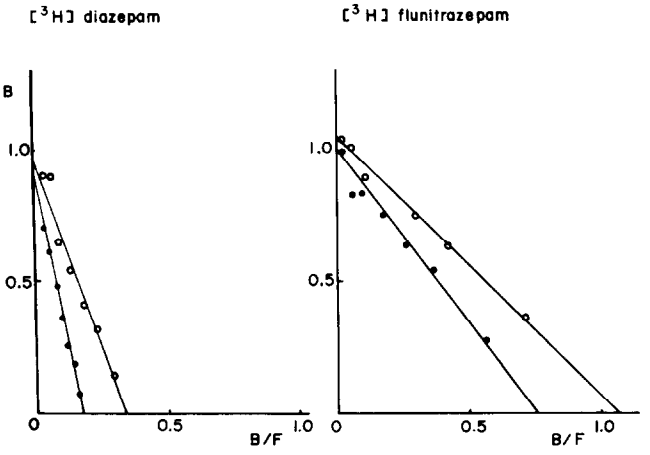

Fig. 1. Scatchard analysis of [^{3}H]diazepam and [^{3}H]flunitrazepam binding to cerebral synaptic membranes. The membrane preparation was incubated with 0.5 nM [^{3}H]diazepam or [^{3}H]flunitrazepam in the presence of varying concentrations of nonradioactive ligand up to 40 nM. The mean value from 3 to 7 separate experiments is shown. The data obtained by the kinetic analysis are as follows: [^{3}H]diazepam binding: K_D = 6.36 nM, B_{max} = 0.94 pmol/mg protein (in the presence of 10^{-5} GABA, K_D = 2.96 nM, B_{max} = 0.98 pmol/mg protein); [^{3}H]flunitrazepam binding: K_D = 1.50 nM, B_{max} = 1.12 pmol/mg protein (in the presence of 10^{-5} M GABA, K_D = 1.03 nM, B_{max} = 1.08 pmol/mg protein). Solid symbols (●——●) and open symbols (○——○) represent the binding in the absence of GABA and in the presence of 10^{-5} M GABA, respectively.

Furthermore, the addition of 10^{-5} M bicuculline did not affect the increase induced by pretreatment with phospholipase C and phospholipase A_2, suggesting that these increases were not due to the activation of a GABA receptor.

Kinetic analysis of [^{3}H]diazepam binding to phospholipase C and phospholipase A_2-treated membranes

The Scatchard analysis (Fig. 2, Table 3) of the binding to the phospholipase C and phospholipase A_2-treated membranes indicated that treatment with phospholipase C induced a statistically significant increase (approx. 30%) of the B_{max} without changing the K_D value, whereas treatment with phospholipase A_2 decreased the K_D by approx. 30% ($P < 0.02$), without affecting the B_{max}. As shown in Table 3, 10^{-5} M GABA also significantly decreased the K_D value for [^{3}H]diazepam binding as in the case of phospholipase A_2 treatment. The K_D for [^{3}H]diazepam binding observed in the phospholipase A_2-treated membrane in the presence of 10^{-5} M GABA, however, tended to be higher than that for control or for the phospholipase C-treated membrane. These results suggest that the decrease in K_D for [^{3}H]diazepam binding induced by GABA may involve a different mechanism compared to that found in the phospholipase A_2-treated membrane.

Effect of phospholipase C and phospholipase A_2 treatments on GABA-induced enhancement of [^{3}H]diazepam binding

Since phospholipase C and phospholipase A_2 had different effects on the benzodiazepine receptor binding measured in the presence of 10^{-5} M GABA, an

Table 1. Effect of various enzymatic treatments of synaptic membranes on [^{3}H]diazepam binding

Treatment	(unit/mg of membraneous protein)	[^{3}H]diazepam bound (% of control)
Trypsin	(50)	51
Pronase	(50)	69
Neuraminidase	(0.12)	113
β-Galactosidase	(0.25)	108
Lipoxygenase	(10)	114
Phospholipase C*	(0.5)	150
Phospholipase A_2*	(0.5)	128
Phospholipase D*	(0.5)	117

* Incubated with 1 mM $CaCl_2$.

Following the preincubation of the membrane with each enzyme at 37°C for 30 min, the preparations were centrifuged at 48,000 *g* for 20 min. The resultant pellets were resuspended with Tris–HCl buffer and used for the binding assay. Each value represents the mean from 3 to 7 separate experiments. The concentration of [^{3}H]diazepam used was 0.5 M.

Table 2. Effect of phospholipase C and phospholipase A_2 treatment of the synaptic membranes on [^{3}H]diazepam and [^{3}H]flunitrazepam binding

	[^{3}H]diazepam bound (% of control)	[^{3}]flunitrazepam bound (% of control)
Muscimol (10^{-5} M) added	247	132
Phospholipase C treated*	143	120
Phospholipase A_2 treated*	129	118

* Following pretreatment of the membrane at 37°C for 30 min with each enzyme (0.5 unit/mg of membraneous protein) in the presence of 1 mM $CaCl_2$, the preparations were centrifuged at 48,000 *g* for 20 min. The resultant pellets were resuspended with Tris–HCl buffer and used for the assay of [^{3}H]diazepam and [^{3}H]flunitrazepam bindings. The concentration of both radiolabelled ligands used was 0.5 nM.

examination was made of the stimulatory effect of various concentrations (10^{-8}–10^{-4} M) of GABA on the [^{3}H]diazepam binding to untreated, phospholipase C-treated and phospholipase A_2-treated membranes. As shown in Figure 3, the sigmoid curves for stimulation were obtained in all three cases. However, the amount of maximal stimulation induced by 10^{-4} M GABA found in the phospholipase A_2-treated membranes was significantly lower than that found in control as well as in the phospholipase C-treated membrane. These results were compatible with the fact that the K_D value observed in the phospholipase A_2-treated membranes in the presence of 10^{-5} M GABA was relatively high (see Table 3).

Effect of the addition of various phospholipids on [^{3}H]diazepam binding to phospholipase C and phospholipase A_2-treated membranes

Since it is well known that both phospholipase C and phospholipase A_2 hydrolyze particular phospholipids, tests were conducted as to whether or not the addition of various phospholipids modified the increase in [^{3}H]diazepam binding found in phospholipase C- and phospholipase A_2-treated membranes. In these experiments, phosphatidyl serine, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidic acid, *O*-phosphoethanolamine, which is known to be the degradation product of phosphatidyl ethanolamine following the action of phospholipase C (Macfarlane and Knight, 1941), and glycerophosphoethanolamine, which is also produced by the action of phospholipase A_2 (Van den Bosch, Postema, de Hass and van Deenen, 1965), were used in a final concentration of 500 μg/ml of membraneous suspension. The addition of phosphatidyl serine and phosphatidic acid completely abolished the increase in the binding induced by phospholipase C and phospholipase A_2 treatment, whereas other phospholipids had little effect on the binding. On the other hand, the addition of phosphatidyl serine and phosphatidic acid to untreated membrane had no effect on the binding. The kinetic analysis of the effect of the addition of phosphatidic acid to phospholipase A_2-treated membrane is shown in Figure 4. Although the K_D value was lowered in phospholipase A_2-treated membranes compared with that found in untreated or phospho-

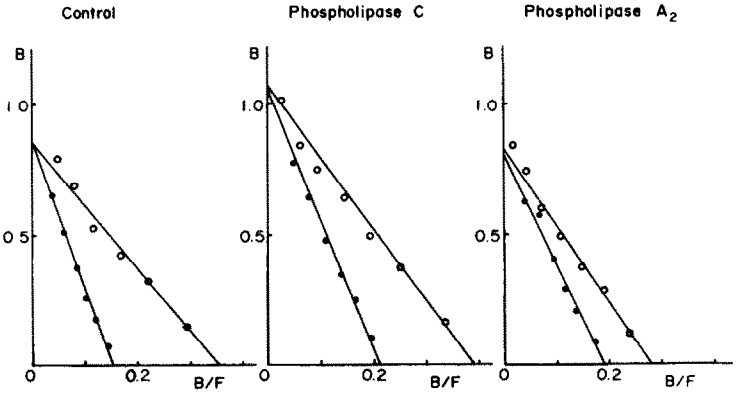

Fig. 2. Scatchard analysis of [^{3}H]diazepam binding to cerebral synaptic membranes treated with phospholipase C and phospholipase A_2. Following pretreatment of the membrane at 37°C for 30 min with phospholipase C and phospholipase A_2 (0.5 unit/mg of membraneous protein), the treated membrane preparations were centrifuged at 48,000 *g* for 20 min. The resultant pellets were resuspended with Tris–HCl buffer and used for [^{3}H]diazepam binding assay as described in the legend of Figure 1. Each point represents the mean from 3–6 separate experiments performed in triplicate. Solid symbols (●——●) and open symboles (○——○) represent the binding in the absence of GABA and in the presence of 10^{-5} M GABA, respectively.

Table 3. Scatchard analysis of [^{3}H]diazepam binding to cerebral synaptic membranes treated with phospholipase C and phospholipase A_2

Treatment	K_D (nM)		B_{max}(pmol/mg protein)	
	−GABA	+GABA (10^{-5} M)	−GABA	+GABA (10^{-5} M)
None	6.48 ± 0.25	2.81 ± 0.14	1.03 ± 0.15	1.01 ± 0.24
Phospholipase C†	6.23 ± 0.33	2.36	1.34 ± 0.09**	1.20
Phospholipase A_2†	4.59 ± 0.32*	3.24 ± 0.04	0.97 ± 0.08	1.05 ± 0.16

*$P < 0.02$, and **$P < 0.05$, compared with each untreated value, respectively.

† Cerebral synaptic membranes were preincubated with each enzyme (0.5 unit/mg of membraneous protein) in the presence of 1 mM $CaCl_2$ ar 37°C for 30 min, and centrifuged at 48,000 *g* for 20 min. The resultant pellet was resuspended with Tris–HCl buffer and used for the assay of [^{3}H]diazepam binding as described in the legend of Figure 1. The data represent the mean ± SEM from 3 to 7 separate experiments.

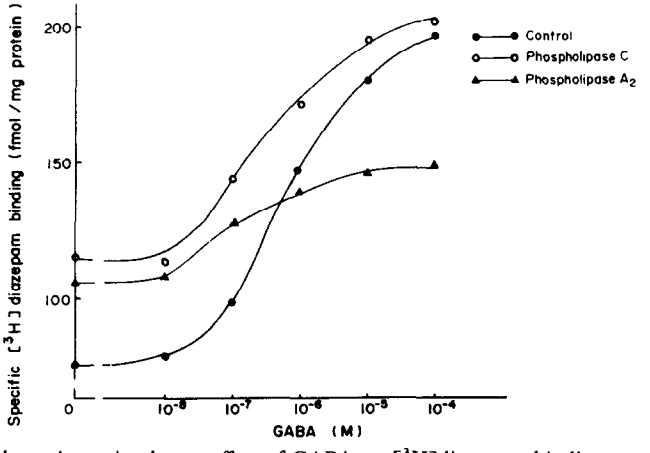

Fig. 3. The dose-dependent stimulatory effect of GABA on [^{3}H]diazepam binding to cerebral synaptic membranes treated with phospholipase C and phospholipase A_2. Each enzymatically treated membrane (see the legend of Fig. 2 for experimental details) was incubated with 0.5 nM [^{3}H]diazepam. Each point represents the mean from at least 2 separate and closely checked experiments performed in triplicate.

	K_D(nM)	B_{max}(pmol/mg prot.)
Control	6.5	0.98
Phospholipase A_2	5.2	0.95
Phospholipase A_2 + Phosphatidic Acid	9.1	0.98

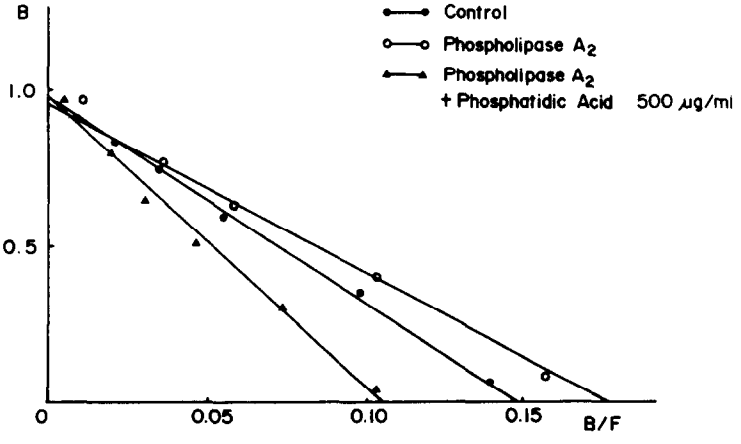

Fig. 4. Effect of addition of phosphatidic acid on [^{3}H]diazepam binding to cerebral synaptic membranes treated with phospholipase A_2. Following the treatment of synaptic membranes with phospholipase A_2, phosphatidic acid was added (for detailed procedure, see Methods). This membrane preparation was incubated with [^{3}H]diazepam as described in the legend of Figure 1. A typical experimental result is shown.

Table 4. Effect of treatment with digitonin, nystatin and polymixin B on [^{3}H]diazepam binding to synaptic membranes

Treatment	[^{3}H]diazepam bound (fmol/mg protein)
None	51.7
0.05% digitonin	58.9
0.5 mM nystatin	61.1
0.5 mM polymixin B	56.3

Digitonin and nystatin were dissolved with a small amount of dimethyl sulfoxide (DMSO). Following pretreatment of the membrane with digitonin, nystatin and polymixin B at 37°C for 30 min, each treated membrane was subjected to the binding assay. The DMSO used in this study had no effect on the binding. The data represent the mean from 2 to 3 separate experiments. The concentration of [^{3}H]diazepam used was 0.5 nM.

lipase C-treated membranes, as previously described, the addition of phosphatidic acid completely abolished the decrease in the K_D value induced by treatment with phospholipase A_2. These results suggest that the removal of phosphatidic acid from the synaptic membrane may be involved in the occurrence of the increased binding of [^{3}H]diazepam induced by phospholipase A_2.

Effect of treatments with digitonin, nystatin and polymixin B on [^{3}H]diazepam binding to the membrane

Pretreatment of the membrane with drugs which interact with cholesterol in the membrane such as digitonin, nystatin and polymixin B (Kinsky, Luse and van Deenen, 1966; Weissman and Sessa, 1967) had no effect on the [^{3}H]diazepam binding.

Effect of phospholipase C and phospholipase A_2 treatments on [^{3}H]flunitrazepam binding to solubilized fraction from the synaptic membrane

In order to examine the possible involvement of membraneous phospholipids in the modulation of binding, [^{3}H]flunitrazepam binding experiments were carried out using the solubilized fraction from cerebral synaptic membranes. It has been found that solubilization of benzodiazepine binding material from synaptic membranes by 1% Nonidet P-40 significantly reduced the binding compared with that found in intact synaptic membranes. Since the binding of [^{3}H]muscimol to the same solubilized material has been found to be increased significantly, possibly due to the removal of the inhibitor for GABA receptor binding (Ito and Kuriyama, unpublished data), it has been considered that the benzodiazepine receptor may be sensitive to solubilization with Nonidet P-40 and may be destroyed, at least in part, during solubilization. The treatment of the solubilized fraction with phospholipase C and phospholipase A_2, however, had no effect on the [^{3}H]flunitrazepam binding (Table 5). These results indicate that the increase in binding induced by phospholipase C and phospholipase A_2 occurs only at the membrane level, and they also suggest that solubilization may remove phospholipids sensitive to enzymatic treatment and/or may dissociate the phospholipid–benzodiazepine receptor interaction which plays an important modulating role in the function of the benzodiazepine receptor in the membrane.

DISCUSSION

It has been well documented that the affinity of flunitrazepam for the benzodiazepine receptor in the brain is much higher than that of diazepam (Braes-

Table 5. Effect of phospholipase C and phospholipase A_2 treatment on [^{3}H]flunitrazepam binding to cerebral membraneous and solubilized fractions

	Treatment	[^{3}H]flunitrazepam bound (% of control)
Membrane fraction	Phospholipase C	120
	Phospholipase A_2	118
Solubilized fraction	Phospholipase C	98.8
	Phospholipase A_2	98.8

Each fraction was preincubated with each enzyme (0.5 unit/mg of membraneous or solubilized protein) in the presence of 1 mM $CaCl_2$ at 37°C for 30 min and used for the assay of [^{3}H]flunitrazepam binding. For preparation of the solubilized fraction, see Methods. The concentration of [^{3}H]flunitrazepam used was 0.5 nM.

trup *et al.*, 1977; Möhler and Okada, 1977a, 1978; Speth *et al.*, 1978a; Squires and Braestrup, 1977). This fact has been also confirmed in this study. It is noteworthy, however, that the stimulatory effect of GABA on [^{3}H]diazepam binding was much greater than that on [^{3}H]flunitrazepam binding as determined in the presence of the same concentration of both radiolabeled ligands. Although the pharmacological significance and the mechanisms underlying these differences are unclear at present, one possibility might be that flunitrazepam has a greater affinity for the benzodiazepine receptor as well as a greater saturation of the receptor than has diazepam; thus, the stimulatory effect of GABA on flunitrazepam binding may be less pronounced than on diazepam binding.

It has been reported that the treatment of synaptic membranes with trypsin surpresses benzodiazepine binding (Möhler and Okada, 1977b). In this study, it has also been found that not only trypsin, but also pronase treatment abolished the binding of [^{3}H]diazepam and [^{3}H]flunitrazepam. These results clearly indicate that the benzodiazepine receptor consists of protein.

One of the important findings in the present study is that treatment of synaptic membranes with phospholipase C and phospholipase A_2 increased significantly the binding of benzodiazepines to the receptor. Scatchard analysis of the increased binding also revealed that treatment with phospholipase C increased the density of the binding site, whereas treatment with phospholipase A_2 increased the affinity of the binding site. Considering that these enzymes hydrolyze particular phospholipids in the synaptic membrane, and that the addition of phospholipids, such as phosphatidyl serine and phosphatidic acid, eliminated the increased binding induced by phospholipase C and phospholipase A_2 treatment, it is highly likely that membraneous phospholipids susceptible to these enzymatic treatments modulate both the density and the affinity of benzodiazepine receptors at synapses. This concept is further supported by the fact that the phospholipase-induced increase in benzodiazepine receptor binding was not affected by bicuculline, a GABA antagonist, whereas disappeared following solubilization of the receptor which may induce the removal of phospholipids. Furthermore, the fact that various agents, which interact with membraneous cholesterol (Kinsky *et al.*, 1966; Weissmann and Sessa, 1967), such as digitonin and polyene antibiotics, had no effect on the [^{3}H]diazepam binding to untreated membranes (Table 4), suggests that membraneous cholesterol may also not participate in the regulation of the function of the benzodiazepine receptor.

It is well known that benzodiazepines have a high lipid solubility and the increase in temperature of incubation significantly diminishes benzodiazepine receptor binding (Braestrup and Squires, 1977; Möhler and Okada, 1977b; Speth, Wastek and Yamamura, 1978). Such facts, coupled with the present findings, suggest that the observed modulating roles of phospholipids on benzodiazepine receptor binding may be exhibited, at least in part, via an alteration in the fluidity of synaptic membranes. Studies on such a possibility and on the specificity of the modulating roles of membraneous phospholipids need to be carried out.

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