Pharmacology Biochemistry & Behavior, Vol. 30, pp. 911–916. Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Asymmetrical Activation of GABA-Gated Chloride Channels in Cerebral Cortex

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Received 4 January 1988

McINTYRE, T. D., R. TRULLAS AND P. SKOLNICK. *Asymmetrical activation of GABA-gated chloride channels in cerebral cortex.* PHARMACOL BIOCHEM BEHAV 30(4) 911–916, 1988.—A pronounced left/right asymmetry of GABA-gated chloride channels was observed in rat cerebral cortex. This asymmetry was manifest as a higher apparent affinity and density of binding sites for the "cage" convulsant [^{35}S]t-butylbicyclophosphorothionate (TBPS) in the right compared to left cortex. Asymmetries in [^{35}S]TBPS binding were not observed in other brain areas, and were restricted to the occipital and entorhinal/pyriform areas of cerebral cortex. Evaluation of other components of the benzodiazepine/GABA receptor chloride ionophore complex in cerebral cortex suggests that this asymmetry is not present in either benzodiazepine receptors or that population of GABA receptors linked to benzodiazepine receptors. Brief restraint-stress significantly increased both the apparent affinity and number of [^{35}S]TBPS binding sites in cortex, but the left/right asymmetry was no longer apparent. These findings indicate a time-dependent, asymmetric response of cortical GABA-gated chloride channels to stress rather than an anatomical hemispheric difference, and suggest that GABA-gated chloride channels may be involved in a differential processing of stressful stimuli by the cerebral hemispheres.

Hemispheric asymmetry Chloride channels GABA [^{35}S]t-butylbicyclophosphorothionate Stress Benzodiazepines

WHILE both direct and correlative evidence suggests that the pharmacological actions of drugs such as benzodiazepines and barbiturates are mediated through the benzodiazepine/GABA receptor chloride ionophore complex ("supramolecular complex") [32,36], the physiological functions of this complex have not been fully elucidated. Recently, however, it was observed that a brief, ambient-temperature swim produced a marked increase in the apparent affinity and number of binding sites for [^{35}S]t-butylbicyclophosphorothionate (TBPS) [15, 16, 34, 35], a picrotoxinin-like "cage" convulsant that binds to GABA-grated chloride channels [29,17]. These findings suggest that the supramolecular complex may be involved in the physiological response to stress.

During the course of characterizing this phenomenon, a marked hemispheric asymmetry of [^{35}S]TBPS binding was observed in rat cerebral cortex. This asymmetry was manifest as a higher apparent affinity and number of binding sites for [^{35}S]TBPS in the right compared to left cerebral cortex, and appeared to be restricted to the occipital and entorhinal/pyriform areas of the cortex. A hemispheric asymmetry in [^{35}S]TBPS binding was not observed in other brain regions (e.g., hippocampus, striatum, pons/medulla and cerebellum), nor was it apparent in either benzodiazepine receptors or that population of GABA receptors coupled to benzodiazepine receptors. This asymmetry was no longer apparent following brief restraint-stress, despite an increase in [^{35}S]TBPS binding to cerebral cortical membranes in stressed animals compared to nonstressed animals. These findings indicate the presence of a functional, rather than an anatomical lateralization of GABA-gated chloride channels which may be related to a differential processing of stressful stimuli by the cerebral hemispheres.

METHOD

Animals

Adult, male Sprague-Dawley rats (Taconic Farms, Germantown, NY; 175–200 g on arrival) were housed for at least 10 days in 36×33×20 cm metal cages (2–5 cage) in an environmental chamber (Consolidated Instruments, Baltimore, MD) which attenuated external olfactory, auditory and visual stimuli [34]. This cabinet (183×92×46 cm) was indirectly illuminated by flourescent lights on a 12 hr light/dark cycle (lights on at 0600). An exhaust fan provided "white" noise. Purina rat chow and water were continuously available.

Animals were removed from their cages and killed by decapitation (a process requiring <10 sec) between 0730 and 0830. No more than one animal per cage was removed on any day to minimize the effect of sequential removal of cohorts from a common cage on [^{35}S]TBPS binding [34]. Animals subjected to restraint-stress were then removed from their cages, transported to another room, and restrained on wooden boards fitted with wire loops for 10 min [19,31].

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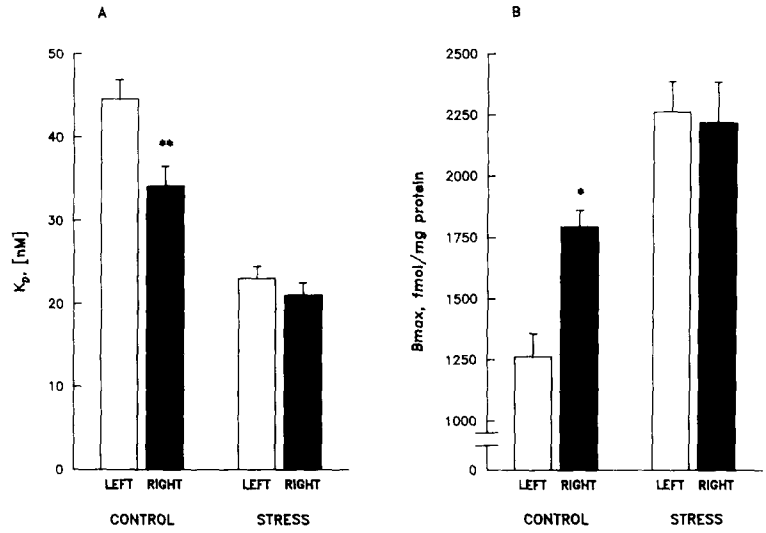

FIG. 1. Cerebral asymmetry in [^{35}]TBPS binding: Effects of restraint-stress. [^{35}S]TBPS binding to well-washed cerebral cortical membranes from left and right hemispheres was assayed as described in the Method section. Values for (A) K_d and (B) B_{max} represent the mean±SEM of five naive (control) and five restraint-stressed rats examined in separate experiments. Symbols: *$p<0.02$; **$p<0.001$, significantly different from corresponding value in left cortex, paired t-test. Stress values were significantly different from corresponding values in control animals, $p<0.05$, Student's t-test. The values obtained by averaging the data from individual left and right cortices are similar to those obtained in whole cortex from animals housed and sacrificed under identical conditions [34,35].

Tissue Preparation

Immediately after decaptiation, brains were rapidly removed and placed in ice-cold 50 mM Tris-HCl buffer (pH 7.4). Brains were dissected using a rat brain atlas [26]. Since parietal cortex is not clearly demarcated in the rat, tissue between the frontal and occipital cortices and dorsal to the temporal cortex was designated parietal cortex. After dissection, tissue was weighed and homogenized for 10 sec in 50 volumes of buffer with a Brinkmann Polytron (setting 6–7).

Radioligand Binding

Radioligand binding to extensively washed membranes was measured as previously described [15,34]. Briefly, homogenates were centrifuged at 20,000×g for 20 min (4°). The tissue pellets were resuspended and centrifuged four more times in 50 volumes of 50 mM Tris-citrate (pH 7.4) buffer containing 100 mM NaCl. The tissue was washed and resuspended a final time in 40 volumes of 50 mM Tris-citrate buffer (pH 7.4). [^{35}S]TBPS binding was assayed (triplicates) in a total volume of 0.5 ml consisting of: 0.3 ml tissue, 0.05 ml of 2 M Nacl, 0.05 ml of [^{35}S]TBPS (final concentration, 6.5 nM; Specific activity 60–120 Ci/mmol), 0.05 ml of TBPS (yielding final concentrations between 6.5 and 166.5 nM), and 0.05 ml of 50 mM Tris-citrate buffer. Nonspecific binding was determined by replacing an equal volume of buffer with 0.05 ml of picrotoxinin (final concentration, 20 μM). Reactions were initiated by the addition of tissue and terminated after 120 min (23°C) by rapid filtration with two 5 ml washes of 50 mM Tris-citrate buffer (pH 7.4) through Whatman GF/B glass fiber filters using a Brandel M-24R filtering manifold (Brandel Instruments, Gaithersburg, MD). [^{3}H]Ro 15-1788 binding to well-washed membranes was assayed (triplicates) in a total volume of 0.5 ml consisting of: 0.3 ml tissue, 0.05 ml 2M NaCl, 0.05 ml [^{3}H]Ro 15-1788 (Specific activity 76.0 Ci/mmol, final concentration, 1.5 nM), and 0.1 ml 50 mM Tris-citrate (pH 7.4) buffer. Nonspecific binding was determined by replacing an equal volume of buffer with 0.05 ml of flunitrazepam (final concentration 10 μM). GABA-enhanced [^{3}H]flunitrazepam (Specific activity, 78.3 Ci/mmol, final concentration, 0.8 nM) binding to the left and right cortex was determined (triplicates) in a total volume of 1.0 ml consisting of: 0.1 ml tissue, 0.1 ml GABA (0.0625–60.0 μM), 0.1 ml [^{3}H]flunitrazepam and 0.7 ml buffer. Nonspecific binding was determined by replacing an equal volume of buffer with 0.1 ml of diazepam (final concentration 10 μM). Incubations (0–4°) were initiated by addition of tissue and terminated after 60 min by rapid filtration through Whatman GF/B filter papers as described. Proteins were determined using the Miller [24] modification of the Lowry technique [21]. Radionuclides and TBPS were obtained from New England Nuclear (Boston, MA). GABA and picrotoxinin were purchased from Sigma Chemical Co. (St. Louis, MO). Benzodiazepines were donated by Hoffmann-LaRoche, Nutley, NJ.

RESULTS

Statistically significant differences, manifest as a higher apparent affinity [K_d values (as the mean±SEM): 44.6±2.4 nM (left cortex) and 34.1±2.3 nM (right cortex), $p<0.001$, paired t-test] and number of binding sites [B_{max} values: 1263±96 fmol/mg protein (left cortex) and 1794±68 fmol/mg protein (right cortex), $p<0.02$, paired t-test] for [^{35}S]TBPS were found in the right compared to the left cerebral cortex

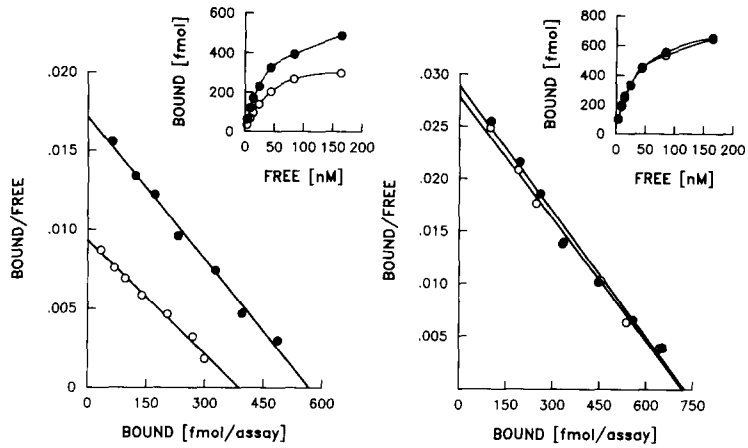

FIG. 2. Representative Scatchard plots of [^{35}S]TBPS binding in left (open circles) and right (closed circles) cerebral cortical membranes from control (left panel) and restraint-stressed (right panel) animals. Scatchard plots were constructed by adding TBPS to [^{35}S]TBPS (6.5 nM) yielding final concentrations between 6.5 and 166.5 nM. Data were analyzed by linear regression with all r>.985. Tissue preparation and assays were conducted as described in the Method section. Inset: Saturation isotherms of this data. In this representative experiment the K_D values of the left and right control and restraint-stressed cortices were: 42.0, 33.2, 25.8, and 25.1 nM, respectively. The corresponding B_{max} values were 1208, 1826, 2692, and 2875 fmol/mg protein, respectively.

TABLE 1

[^{35}S]TBPS BINDING IN THE CENTRAL NERVOUS SYSTEM: LEFT AND RIGHT HEMISPHERIC DIFFERENCES

Brain Area	Left	Right	p
Cortex	157 ± 13	291 ± 28	<0.05
Hippocampus	171 ± 22	184 ± 26	NS
Striatum	201 ± 12	217 ± 11	NS
Pons/Medulla	41 ± 2	34 ± 5	NS
Cerebellum	146 ± 8	146 ± 9	NS

[^{35}S]TBPS binding to well-washed membranes derived from left and right brain areas was assayed as described in the Method section. Values are expressed in fmol/mg protein, and represent mean ± SEM of three separate experiments for each area using different animals each time (n=3). Data were evaluated with a paired t-test. The percent difference between the left and right cortices was 85%. The radioligand concentration used in these experiments was 6.5 nM.

TABLE 2

[^{35}S]TBPS BINDING IN DIFFERENT AREAS OF THE CEREBRAL CORTEX: LEFT AND RIGHT HEMISPHERIC DIFFERENCES

Cortical Area	Left	Right	p
Occipital	79 ± 17	132 ± 22	<0.01
Frontal	204 ± 29	228 ± 40	NS
Temporal	304 ± 27	275 ± 35	NS
Parietal	252 ± 31	250 ± 32	NS
Entorhinal/Pyriform	321 ± 48	400 ± 37	<0.05

[^{35}S]TBPS binding to well-washed membranes derived from left and right cortical areas was assayed as described in the Method section. Values are expressed in fmol/mg protein, and represent mean ± SEM of six separate experiments for each area using different animals each time (n=6). Data were evaluated with a paired t-test. The percent differences observed between the left and right occipital, and entorhinal/pyriform cortices were 67% and 25% respectively. This 92% difference closely approximates the 85% asymmetry observed in whole cortex in a different set of experiments (Table 1). Radioligand concentration in these experiments was ≈6.5 nM.

(Figs. 1 and 2). Within the cerebral cortex, statistically significant left/right asymmetries in [^{35}S]TBPS binding were found in the occipital and (pooled) entorhinal/pyriform areas (Table 2). Left/right asymmetries in [^{35}S]TBPS binding were not observed in other brain areas, including hippocampus, striatum, pons/medulla, and cerebellum (Table 1).

In order to determine whether a hemispheric asymmetry was present in other components of the supramolecular complex, [^{3}H]Ro 15-1788 binding to benzodiazepine receptors was examined in several brain areas, and the potency and efficacy of GABA to enhance [^{3}H]flunitrazepam binding determined in cerebral cortex. In contrast to the marked asymmetry in [^{35}S]TBPS binding present in cerebral cortex, no asymmetries in [^{3}H]Ro 15-1788 binding were observed in either cortex, hippocampus, striatum, pons/medulla or cerebellum (Table 3). Furthermore, neither the potency nor efficacy of GABA to enhance [^{3}H]flunitrazepam binding in well-washed membranes were different between left and right cerebral cortex (Table 4).

As has been reported following a brief, ambient-temperature swim [15, 16, 34], restraint-stress produced a statistically significant (p<0.01, Student's t-test) reduction in the K_d of [^{35}S]TBPS [K_d values: 23.1±1.2 nM (left cortex)

TABLE 3

[^{3}H]RO 15-1788 BINDING IN DIFFERENT LEFT AND RIGHT BRAIN AREAS

Brain Area	Left	Right	p
Cortex	557 ± 38	561 ± 60	NS
Hippocampus	385 ± 32	336 ± 52	NS
Striatum	260 ± 26	283 ± 44	NS
Pons/Medulla	142 ± 27	124 ± 4	NS
Cerebellum	287 ± 19	266 ± 21	NS

Values are expressed in fmol/mg protein, and represent mean ± SEM of three separate experiments for each area using different animals each time (n=3). The radioligand concentration in these experiments was ≈1.5 nM.

TABLE 4

GABA-ENHANCED [^{3}H]FLUNITRAZEPAM BINDING IN CONTROL RATS: COMPARISON OF LEFT AND RIGHT CEREBRAL CORTEX

	[^{3}H]Flunitrazepam (fmol/mg protein)	+GABA	
		EC_{50} (μM)	E_{max}
Left	63 ± 4	0.38 ± 0.01	58.3 ± 2.1%
Right	68 ± 3	0.39 ± 0.02	58.8 ± 2.6%

[^{3}H]Flunitrazepam binding to well-washed cortical membranes was determined as described in the Method section. Values for EC_{50} and E_{max} represent mean ± SEM of four separate experiments using different animals for each experiment (n=4), and were estimated by iterative fitting to a sigmoidal function, $f(x) = A/1 + (b^c/x^c)$, modified from Spencer and Traber [28] on *MLAB* (NIH).

and 22.0±1.5 nM (right cortex)], and a concomitant increase ($p<0.05$, Student's t-test) in the B_{max} for this radioligand [B_{max} values: 2264±122 fmol/mg protein (left cortex) and 2220±165 fmol/mg protein (right cortex)] (Fig. 1). However, the hemispheric asymmetry in [^{35}S]TBPS binding observed in cortex of nonrestraint-stressed animals was no longer apparent (Fig. 1 and 2).

DISCUSSION

A marked left/right asymmetry in the apparent affinity and number of [^{35}S]TBPS binding sites has been described in rat cerebral cortex. Both electrophysiological and biochemical studies [1, 17, 29] suggest that "cage" convulsants like picrotoxinin and TBPS bind to GABA-gated chloride channels, and that the apparent affinity and number of [^{35}S]TBPS binding sites may be related to the permeability characteristics and number of "open" GABA-gated chloride channels, respectively [17]. While the lateralization of a number of neurotransmitters, their biosynthetic enzymes, and receptors have been reported [12–14, 23, 30, 39], this is the first evidence for an asymmetry of hormone-gated ion channels in the central nervous system. Since GABA-gated chloride channels are one component of a supramolecular complex containing discrete recognition sites for benzodiazepines and GABA (cf., [27, 32, 36] for review), radioligand binding studies were performed to determine whether this asymmetry was common to other components of the supramolecular complex. However, it appears that neither benzodiazepine receptors (as assessed by the binding of a specific, high affinity ligand of benzodiazepine receptors, [^{3}H]RO 15-1788 [18,25], nor that population of GABA-receptors coupled to benzodiazepine receptors (assessed by examining the potency and efficacy of GABA to enhance [^{3}H]flunitrazepam binding [5,8]) are asymmetrically distributed in cerebral cortex. Although differences in [^{35}S]TBPS binding between both brain areas and cortical laminae have been reported using autoradiographic techniques [9, 22, 38], to our knowledge, hemispheric asymmetries have not been described. However, these autoradiographic studies did not attempt to quantify hemispheric asymmetries. Furthermore, since stress can eliminate hemispheric differences in [^{35}S]TBPS binding (Figs. 1 and 2), the behavioral state of the animals used in those studies could contribute to an apparent lack of asymmetry.

The hemispheric asymmetry in [^{35}S]TBPS binding was confined to the occipital and entorhinal/pyriform cortices, areas of the cerebral cortex involved in sensory processing (Table 2). This observation was consistent with the changes in [^{35}S]TBPS binding observed in whole cortex (Table 1). Thus an 85% difference in [^{35}S]TBPS binding was observed between left and right whole cortex (Table 1), while in separate studies investigating the regions responsible for this difference, a 92% left/right difference was obtained by summing the percent asymmetry between individual areas (Table 2). Since perturbation of the environment (e.g., sequential removal of cohorts from a common cage or a brief, ambient-temperature swim [34]) can produce an increased affinity and number of [^{35}S]TBPS binding sites, the left/right asymmetry could be attributable to the sensory stimuli associated with removal and sacrifice of the animal. Thus, the persistence of a cortical asymmetry in animals subjected to stress would indicate an anatomical lateralization, whereas if the asymmetry was no longer apparent after stress, it could be inferred that there was a differential temporal response of GABA-gated chloride channels to removal of the animal from its home cage environment. In order to differentiate between these possibilities, rats were subjected to a brief period of restraint-stress. Restraint, like an ambient-temperature swim [34], produced a significant reduction in the K_d and a concomitant increase in the B_{max} of [^{35}S]TBPS in cortical membranes of both hemispheres. However, the asymmetry observed in nonrestraint-stressed (control) animals was no longer apparent (Fig. 1). Furthermore, sequential removal of three rats from the same home cage gradually eliminated asymmetrical differences in both the K_d and B_{max} of [^{35}S]TBPS (data not shown). These findings suggest that the cerebral asymmetry in GABA-gated chloride channels may be related to hemispheric differences in processing sensory information (provoked by removal and sacrifice of the animal), rather than an anatomical asymmetry. The presence of an asymmetry in [^{35}S]TBPS binding in animals removed from their home environment and sacrificed in <10 sec indicates that the mechanism(s) responsible for these alterations in GABA-gated chloride channels is responsive to even minor environmental perturbation, and responds at a more rapid rate than previously reported [34]. It has been proposed that the release of an endogenous ligand with benzodiazepine-like actions may be responsible for these stress-induced changes in [^{35}S]TBPS binding [35]. This possibility is currently under investigation.

While the physiological significance of this differential

activation of GABA-gated chloride channels is unknown, it is consistent with evidence suggesting a hemispheric specialization of stimulus processing by the cerebral hemispheres [10]. Functional asymmetries of the cerebral hemispheres have been well documented in both humans and animals [2, 6, 7, 10, 11]. A functional specialization of the cerebral hemispheres specifically mediated by GABA-gated chloride channels is supported by clinical studies demonstrating that unilateral, intracarotid administration of amobarbital (a ligand acting at sites on the supramolecular complex [20]) produces either euphoria or depression depending on the side injected [33,37]. In addition, it has also been demonstrated using both EEG and PET techniques that marked differences between left and right occiptal cortices are apparent following benzodiazepine administration in clinically anxious patients [3,4]. For instance, patients diagnosed (DSM III) as having generalized anxiety disorder and administered chlorazepate for 21 days had a decreased glucose metabolism in the right compared to left occipital cortex [4]. Therefore, the time-dependent, functional asymmetry in GABA-gated chloride channels reported here may reflect an important physiological mechanism necessary for responding to anxiety-inducing perturbations of the environment.

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