of these SH-compounds, the Cu^{2+} is reduced by the SH-compounds as well as by O_2^- and then reoxidized by O_2^-, by which the redox cycle between Cu^{2+} and Cu^+ is accelerated. Consequently, the dismutation of O_2^- by the enzyme is enhanced. The present results also suggest that these SH-compounds might participate in protecting the cell against oxygen toxicity in vivo by activating Cu, Zn-SOD, in addition to their function of directly scavenging O_2^- which has been found in vitro[8,9,11,12].

1 Acknowledgment. The authors wish to thank Dr C. Brünger for reading the manuscript.
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0014-4754/85/111416-04$1.50 + 0.20/0

3[H]-Sulpiride labels mesolimbic non-dopaminergic sites that bind antidepressant drugs[1]

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Summary. 3[H]-(−)-Sulpiride and 3[H]-spiperone binding was compared in rat amygdala, nucleus accumbens and striatum, using (+/−)-sulpiride to define specific binding. 3[H]-(−)-Sulpiride bound to twice as many sites in amygdala and nucleus accumbens as 3[H]-spiperone. 3[H]-(−)-Sulpiride binding was directed to these additional sites by using 1 μM spiperone to mask dopaminergic binding. The binding of 3[H]-(−)-sulpiride to these sites was high affinity, reversible, Na^+-dependent, but not stereospecific. Metoclopramide, tiapride and antidepressant medications, but not other neuroleptics, ADTN, or serotonin displaced 3[H]-(−)-sulpiride binding to these sites. These data suggest that 3[H]-(−)-sulpiride labels mesolimbic sites other than dopamine receptors which may mediate antidepressant effects.
Key words. Sulpiride; spiperone; antidepressants; substituted benzamide; alprazolam.

Dopamine (DA) receptors are present in several brain areas. Because of efforts to measure DA receptor binding in mesolimbic brain areas as well as striatum, we compared the binding of 3[H]-(−)-sulpiride and 3[H]-spiperone in amygdala, nucleus accumbens, and striatum of rat brain. Both of these ligands bind with high affinity to post-synaptic DA D2 (i.e. non-adenyl cyclase coupled) receptors. In addition, 3[H]-(−)-sulpiride as well as 3[H]-spiperone binds pre-synaptic DA receptors on corticostriatal glutamate neurons in rat[3–5]. Other investigators have shown that in striatum these two ligands bind the same number of sites when sulpiride is used as the counterligand to define specific binding[6]. However, we now report that in amygdala and nucleus accumbens, 3[H]-(−)-sulpiride binds additional sites distinct from DA receptors bound by 3[H]-spiperone. Antidepressants and other substituted benzamides, but not other neuroleptics, ADTN, or serotonin demonstrated high affinity for these sites.

Methods. Male Sprague-Dawley rats (150 g, Simonsen Laboratories, Gilroy, CA) were sacrificed by decapitation and their brains were removed for receptor binding assays[7]. Frontal cortex, amygdala, nucleus accumbens, and striatum were dissected on ice and homogenized in ice-cold 50 mM Tris buffer (pH 7.6), containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 50 μM tranylcypromine, and 0.1% ascorbate, using a Brinkmann Polytron homogenizer (setting 5, 10 s). The homogenates were centrifuged twice at 50,000 × g with resuspension in fresh buffer. The total assay volume of 1.0 ml contained 0.3–0.8 mg of protein, measured using the Biorad protein assay[8], depending on the brain area assayed. 3[H]-(−)-Sulpiride (78.2 Ci/mmole) and 3[H]-spiperone (22.8 Ci/mmole) were obtained from New England Nuclear (Cambridge, MA). Bound and unbound ligand were separated by rapid filtration through Whatman GF-B filters followed by two 5-ml washes of buffer. The filters were counted after the addition of 10 ml Aquasol-2 scintillation cocktail. For experiments where 3[H]-(−)-sulpiride binding was directed to non-dopaminergic sites 1.0 μM spiperone was added to the assay mixture. The reversibility of this component of 3[H]-(−)-sulpiride binding was assessed by measuring the residual binding of 10 nM 3[H]-(−)-sulpiride in triplicate after the addition of 10 μM (+/−)-sulpiride at various time points (0.5–20 min), and its Na^+ dependence by comparing binding in the presence or absence of 140 mM NaCl.

Results. Table 1 summarizes the binding characteristics of 3[H]-spiperone, 3[H]-(−)-sulpiride, and 3[H]-(−)-sulpiride in the presence of 1.0 μM spiperone, in three brain areas. No specific binding was detected in frontal cortex for any of the three binding conditions. 3[H]-(−)-sulpiride binding was performed in the presence of 1 μM spiperone in the amygdala and nucleus accum-

bens because of the discrepancy between 3[H]-(−)-sulpiride and 3[H]-spiperone B(max) values. 1 μM was chosen as the spiperone concentration needed to mask the dopaminergic component of 3[H]-(−)-sulpiride binding because spiperone competition experiments in amygdala and nucleus accumbens yielded biphasic curves. Hill coefficients were much less than 1.0 (in amygdala, 0.20; in nucleus accumbens, 0.26), suggesting two sites. Plateaus in spiperone competition curves for both brain areas occurred in the 0.3–3.0 μm range. Interestingly, the discrepancy in B(max) values between 3[H]-spiperone and 3[H]-(−)-sulpiride in amygdala and nucleus accumbens equalled the number of 3[H]-(−)-sulpiride binding sites in the presence of 1 μM spiperone. In striatum, we also found that 3[H]-spiperone and 3[H]-(−)-sulpiride bound an equivalent number of sites.

Hill coefficients for 3[H]-(−)-sulpiride binding in amygdala and nucleus accumbens in the presence of 1 μM spiperone were 0.90 ± 0.01 and 0.98 ± 0.01 (mean of three separate determinations ± SEM), respectively, which suggested that this additional binding now represented a single site. Dissociation of 3[H]-(−)-sulpiride from this additional site was linear; the dissociation half-life was 1.7 min in amygdala and 1.7 min in nucleus accumbens. Binding of 10 nM 3[H]-(−)-sulpiride to these additional sites was reduced in the absence of NaCl by 74% in amygdala and 62% in nucleus accumbens (percent differences calculated from means of 4–8 determinations, SEM < 10%). Table 2 summarizes the results of competition binding experiments to characterize the additional 3[H]-(−)-sulpiride site in amygdala and nucleus accumbens. Several neuroleptics, tricyclic antidepressants, serotonergic ligands, and benzodiazepines were compared. Potent displacers of 3[H]-(−)-sulpiride included other benzamides, four tricyclic antidepressants, and an atypical benzodiazepine. Several pre- and post-synaptic dopaminergic ligands and serotonergic ligands were ineffective displacers.

Discussion. In striatum, the binding of 3[H]-(−)-sulpiride to post-synaptic D2 and corticostriate pre-synaptic DA receptors is reversible, stereospecific, and high-affinity (K(d) 7–17 nM)[1,4,5,9–12]. Spiperone is known to bind these sites and S2 serotonin receptors[13]. Furthermore, 3[H]-(−)-sulpiride has been shown to be highly selective for these DA receptors. It does not inhibit DA-stimulated adenyl cyclase (presumably by binding D1 receptors), and has no affinity for muscarinic acetylcholine, serotonin, alpha-1-adrenergic, alpha-2-adrenergic, or histamine receptors[5]. Also, because we found no binding of 3[H]-(−)-sulpiride in the frontal cortex, it appears that this ligand has no affinity for beta-adrenergic receptors or reuptake sites, since they are both prevalent in this brain area. 3[H]-Spiperone is known to bind to both striatal DA sites and S2 serotonin receptors.

However, our data suggest that 3[H]-(−)-sulpiride has additional binding sites in two mesolimbic structures apart from expected DA receptors bound by 3[H]-spiperone. This binding was of high affinity, reversible, and apparently represented a single site. It was also Na^+-dependent, as is sulpiride binding to DA receptors[14–17]. However, it was not stereospecific since both enantiomers of sulpiride displaced it equally.

These non-dopaminergic 3[H]-(−)-sulpiride sites may represent non-stereospecific but saturable sites (NSS) that occur with any 3[H]-ligand when the cold ligand is used itself to define specific binding[13]. However, this is unlikely since we found the additional 3[H]-(−)-sulpiride sites only in mesolimbic structures. Also, to eliminate this possibility, we performed additional 3[H]-(−)-sulpiride binding experiments in the presence of 1 μM spiperone, using 10 μM metoclopramide rather than (±)-sulpiride to define specific binding. However, despite this counterligand substitution, the B(max) of the additional 3[H]-(−)-sulpiride sites in nucleus accumbens (0.254 pmoles /ml protein) and amygdala (0.283 pmoles/mg protein) remained unchanged.

Two other substituted benzamides, tiapride and metoclopramide, and four tricyclic antidepressants demonstrated high affinity for the non-dopaminergic 3[H]-(−)-sulpiride binding sites. Other neuroleptics, ADTN, and serotonin ligands had no affinity for these sites. Interestingly, alprazolam, a triazolo-benzodiazepine with tentative antidepressant as well as anxiolytic efficacy[18], also demonstrated high binding affinity, while diazepam did not.

The high affinity of both (−)- and (+)-sulpiride for these sites, suggested by their equipotent displacement of 3[H]-(−)-sulpiride, seems disconcerting since (−)-sulpiride is generally considered to be the active enantiomer of the drug. However, while most of the neuroleptic-like behavioral and binding properties of this drug are limited to (−)-sulpiride[5,19], (+)-sulpiride also has pharmacological effects. Both (−)-sulpiride and (+)-sulpiride increase prolactin secretion[20–22] and bind renal dopamine receptors[23]. Both (+)- and (−)-sulpiride elevate systolic blood pressure[21]. (+)-Sulpiride, like dopamine agonists, inhibits gastric acid secretion, while having no effect on circulating levels of gastrin[24]. But most relevant to our data, (+)-sulpiride has been reported to be more active than (−)-sulpiride in reducing immobility in the forced swimming test in rats, an animal model for antidepressant efficacy[25].

Table 1. Comparison of 3[H]-spiperone and 3[H]-(−)-sulpiride in three brain areas, and isolation of non-dopaminergic mesolimbic 3[H]-(−)-sulpiride sites

	3[H]-Spiperone		3[H]-(−)-Sulpiride		3[H]-(−)-Sulpiride with 1 μM spiperone	
	K(d) (nM)	B(max) (pmol/mg)	K(d) (nM)	B(max) (pmol/mg)	K(d) (nM)	B(max) (pmoles/mg)
Amygdala	0.12 ± 0.02	0.22 ± 0.01	13.5 ± 4.3	0.54 ± 0.01	15.4 ± 4.6	0.29 ± 0.02
Nucleus accumbens	0.12 ± 0.01	0.21 ± 0.01	13.8 ± 4.2	0.52 ± 0.04	13.1 ± 3.8	0.29 ± 0.02
Striatum	0.20 ± 0.02	0.66 ± 0.02	17.8 ± 1.0	0.68 ± 0.01	No specific binding	

To obtain K(d) and B(max) values by linear regression, Scatchard plots were determined in triplicate with eight data points using 0.05–2.0 nM 3[H]-spiperone or 1.0–20.0 nM 3[H]-(−)-sulpiride. Each data point was also determined in triplicate, using 10 μM (±)-sulpiride to define specific binding.

Table 2. Displacement of mesolimbic non-dopaminergic 3[H]-(−)-sulpiride binding in the presence of 1.0 μM spiperone

Displacer	Amygdala		Nucleus accumbens	
	IC 50 (nM)	K(i) (nM)	IC 50 (nM)	K(i) (nM)
(+/−)-Sulpiride	15	9	13	7
(−)-Sulpiride	22	13	12	7
(+)-Sulpiride	25	13	16	9
Tiapride	70	42	100	57
Metoclopramide	150	91	50	28
Fluphenazine	> 10,000	> 6000	> 10,000	> 6000
cis-Thiothixene	> 10,000	> 6000	> 10,000	> 6000
Thioridazine	> 10,000	> 6000	> 10,000	> 6000
cis-Flupenthixol	> 10,000	> 6000	> 10,000	> 6000
trans-Flupenthixol	> 10,000	> 6000	> 10,000	> 6000
Chlorpromazine	> 10,000	> 6000	> 10,000	> 6000
ADTN	> 10,000	> 6000	> 10,000	> 6000
Serotonin	> 10,000	> 6000	> 10,000	> 6000
Cinnanserin	> 10,000	> 6000	> 10,000	> 6000
Imipramine	50	30	150	85
Desipramine	300	182	200	114
Amitriptyline	240	146	570	324
Nortriptyline	48	29	65	37
Alprazolam	120	73	500	284
Diazepam	> 10,000	> 6000	> 10,000	> 6000
Phenobarbital	> 10,000	> 6000	> 10,000	> 6000

IC 50 values were obtained from competition curves using 12 data points. Binding at each data point was performed in triplicate. K(i) values calculated using the method of Cheng and Prusoff[30].

Sulpiride is clinically used as a drug with mixed antipsychotic and antidepressant effects[5]. There is one open-label study[26] and one double-blind study comparing sulpiride with amitriptyline[27] that documents sulpiride's antidepressant effects. Unfortunately, the investigators in both these studies did not note whether optically active or racemic drug was administered. Interestingly, a single case of mania caused by metoclopramide has been recently reported[28]. Since induction of mania is a phenomenon usually observed with antidepressants, this suggests that other substituted benzamides may also have antidepressant effects.

Because antidepressants demonstrated high affinity for the mesolimbic non-dopaminergic 3[H]-(−)-sulpiride sites described here, binding of sulpiride to these sites may mediate the antidepressant effects of this drug. In fact, binding of tricyclic and other antidepressants to such sites may be relevant for their therapeutic effects. This latter hypothesis is supported by the fact that IC 50 values obtained for the tricyclic antidepressants were consistent with their therapeutic serum levels[29]. Further studies are needed to associate these mesolimbic 3[H]-(−)-sulpiride sites with a particular neurotransmitter.

1 We wish to acknowledge the help of Dr Roland Ciaranello, who suggested that tricyclic antidepressants might displace the additional 3[H]-(−)-sulpiride binding, and Drs Mark Hambin and Philip Seeman, who reviewed the manuscript. We thank the following pharmaceutical companies for providing drug reagents: (±)-sulpiride – Delagrange; (+)- (−)-sulpiride – Ravizza; diazepam – Roche; alprazolam – Upjohn; imipramine – Ciba; Desipramine – USV Pharmaceutical; amitriptyline – Merck, Sharp and Dohme; nortriptyline – Lilly: cinnanserin and fluphenazine – Squibb; cis-thiothixene – Pfizer; thioridazine – Sandoz; cis- and trans-flupenthixol – H. Lundbeck; chlorpromazine – Smith, Kline and French; spiroperidol – Janssen; metoclopramide – A.H. Robbins; and tiapride – Essex Pharma A/S. This work was supported by NIMH grant MH-03030 (LEH), by a VA Research Associate Award to JGC, and by the Research Service of the Veterans Administration.

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0014-4754/85/111419-03$1.50 + 0.20/0

Lowering of liver acetaldehyde but not ethanol concentrations by pretreatment with taurine in ethanol-loaded rats

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Summary. A rise in blood and liver acetaldehyde concentrations following ethanol loading (1.5 g/kg b.wt) was significantly reduced when rats were pretreated orally with taurine (0.5 g/kg), a potent in vitro activator of yeast aldehyde dehydrogenase. This taurine pretreatment produced a 4-fold increase in liver taurine content.
Key words. Taurine; acetaldehyde; ethanol; ALDH.

We have reported that the oral administration of a clinical dose of pantethine results in a significant inhibition of blood acetaldehyde elevation following alcohol administration to nonflushing, but not to flushing subjects[1]. The pantethine action is based upon the interaction of hepatic aldehyde dehydrogenase and pantethine-related metabolites formed in the liver, such as taurine, D-pantetheine, coenzyme A and D-pantothanate. During these studies, we observed that among these metabolites taurine most potently activates yeast ALDH activity in vitro[2]. The present study was conducted to investigate whether or not an oral administration of taurine could directly diminish a rise in blood acetaldehyde levels following ethanol loading of rats.

Materials and methods. Male Sprague-Dawley rats, weighing 200–260 g each, were starved overnight. A 15% ethanol solution was administered intragastrically at a dose of 1.5 g/kg b.wt. Taurine (Sigma Chemical Co., St Louis) was administered intragastrically in the early morning 30 min before ethanol administration at a dose of 0.5 g/kg b.wt. Rats were killed by exsangui-