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Aminomethyl-1,2,4-benzothiadiazines as Potential Analogues of γ-Aminobutyric Acid. Unexpected Discovery of a Taurine Antagonist

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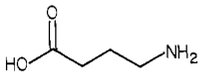
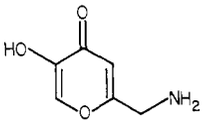
A series of 6- and 8-(aminomethyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides has been synthesized and tested for interaction with various GABA systems. None of the compounds showed significant GABA-mimetic properties, but unexpectedly, compound **7** [6-(aminomethyl)-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide] possessed the properties of a selective antagonist of taurine, as measured by the antagonism of taurine-induced inhibition of rat cerebellar Purkinje firing.

Ever since the discovery of its presence in the nervous system,[2] γ-aminobutyric acid (GABA) has been the object



GABA



kojic amine

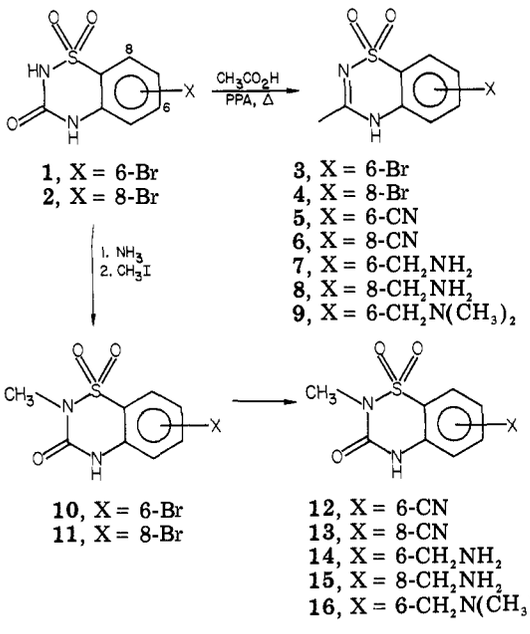
of intense and continuous research in efforts to unravel its various functions, its role in the pharmacological action of a number of drugs acting through the central nervous system (CNS), and as a basis for the development of new and useful drugs.[3] One of the most fruitful approaches to the study of the action of GABA has been the synthesis of relatively rigid molecules of known, restricted conformations as discussed recently by O'Donnell, Johnson, and Azzaro.[4]

We have previously reported on the synthesis and muscle-relaxant activity of the planar GABA analogue kojic amine,[5] and others have reported on further aspects of its pharmacology and mechanism of action.[6,7] In the present article we report the synthesis of a series of aminomethyl-1,2,4-benzothiadiazines (**7–9**, **14–16**; Scheme I), which like kojic amine, incorporate the elements of an acidic and a basic center in a rigid, planar framework.

Of further encouragement in considering these structures, in analogy with kojic amine, was the fact that the 1,2,4-benzothiadiazine structure has a weakly acidic pK_a value of about 9,[8] similar to the value of 8 found for kojic amine.[5] In addition, several lines of evidence point to the tautomer with the proton located on N(4) as being the predominant form in solution,[9,10] which places the acidic and basic centers in the target compounds the same distance apart as found in GABA.

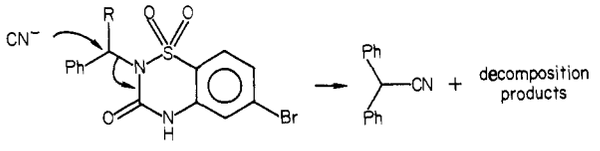
Chemistry. We have recently reported[11] a new one-step synthesis of 1,2,4-benzothiadiazines, which provided the basis for the present syntheses. At that time we made the incidental observation that the 2*H*-1,2,4-benzothiadiazine-3(4*H*)-one 1,1-dioxide system could be converted directly to the 3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide structure by simply heating in a mixture of acetic acid and hydrochloric acid. This "side reaction" was turned to advantage when it was found that the bromo

Scheme I



1, X = 6-Br
2, X = 8-Br
3, X = 6-Br
4, X = 8-Br
5, X = 6-CN
6, X = 8-CN
7, X = 6-CH_2NH_2
8, X = 8-CH_2NH_2
9, X = 6-$CH_2N(CH_3)_2$
10, X = 6-Br
11, X = 8-Br
12, X = 6-CN
13, X = 8-CN
14, X = 6-CH_2NH_2
15, X = 8-CH_2NH_2
16, X = 6-$CH_2N(CH_3)_2$

Scheme II



17, R = H
18, R = Ph

compounds **1** and **2** (as a mixture) could be converted to a mixture of **3** and **4** (Scheme I) in 65% yield by heating

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with a mixture of acetic acid in polyphosphoric acid. The resulting mixture of bromides was converted to the nitriles **5** and **6** in 70% yield with CuCN in DMF. The nitriles were separated by chromatography and reduced to the desired amines **7** and **8** over PtO_2 in ethanol containing chloroform.[12] Compound **9** was prepared by standard Clark-Eschweiler conditions.

Several attempts to displace the bromine atom of **1** and **2** by cyanide failed, the reaction conditions causing extensive decomposition, and no identifiable products could be isolated. However, the 2-methyl compounds, **10** and **11**, were stable to the reaction with CuCN and were converted to the target structures **14–16** without incident.

Several attempts were made to prepare the compound corresponding to **14** lacking the 2-methyl group. The 2-benzyl compound, **17**, could be readily prepared, but it was not possible to remove the benzyl group by catalytic hydrogenation. When the 2-benzhydryl compound, **18**, was treated with CuCN in DMF, the only identifiable product was diphenylacetonitrile, resulting from nucleophilic attack by cyanide at the benzhydryl carbon (Scheme II).

Yudis obtained a value of 9.2 for the pK_a of diazoxide in DMF–water (1:2),[8] and we have measured the pK_a's of several representative compounds in our series for comparison purposes. For solubility reasons, we had to obtain them in Me_2SO–water (1:1), and in this medium, diazoxide had a pK_a of 8.75. Compounds **7** and **8** titrated for 2 equiv of acid across the pH range of 7.5–11 with no observable break in the curve. Taking the pH values at 25 and 75% of the titration curves gave estimated pK_a values of 8.5 and 10.1 for **7** and 8.2 and 10.0 for **8**; the first value in each case is tentatively assigned to the proton on N(4) and the second value to the amine. The titration curve of compound **14**, on the other hand, had observable inflection points at 8.1 and 10.4, which we again assign as the pK_a values of the acidic proton on N(4) and the primary amine, respectively.

Results and Discussion

Compounds **7–9**, and **14–16** were tested for inhibition or stimulation in the following in vivo neurochemical assays using rat brain tissue: GABA transaminase, GABA uptake, GABA release, and [^{3}H]muscimol binding of the GABA agonist. In the synaptosomal GABA transaminase assay at 100 μM final concentration, all six compounds produced an inhibition of succinate formation of between 20 and 30% as compared to the 60% inhibition seen with the transaminase inhibitor, aminooxyacetic acid. A similar 30% maximal effect at 100 μM for these compounds was seen for synaptosomal GABA uptake as compared to a 70% reduction by the uptake inhibitor nipecotic acid at the same concentration. None of the compounds, again at 100 μM final concentration, had any effect on [^{3}H]-GABA release from rat cortical synaptosomes prelabeled with the radiolabeled amino acid, while the IC_{50} for all six compounds against 2 nM [^{3}H]muscimol in rat brain crude synaptic membranes was greater than 20 μM. GABA itself in the latter system had an IC_{50} of 50 nM.

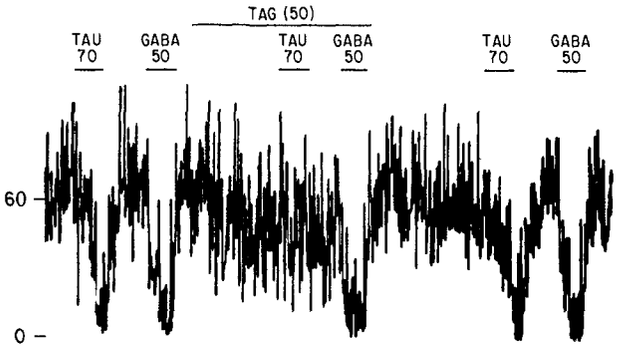
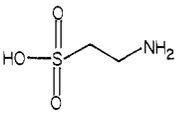


Figure 1. Antagonism of the inhibitory effects of taurine, but not GABA, by compound **7** (TAG) on the spontaneous firing of a cerebellar Purkinje cell. Ordinate represents firing rate in action potential per second. Horizontal line above the rate-meter trace represents periods of drug ejection (taurine and GABA applied for 25 s), and the numbers represent current ejection intensities in nanoamperes.

Thus, none of the target structures showed any significant degree of interaction with physiologically relevant GABA systems, indicating an absence of significant GABA-mimetic or antagonist properties.

As noted initially, in all of the compounds reported here, the distance between the basic nitrogen and the acidic center is almost identical with the distance between the amine and carboxyl groups of GABA in its extended conformation (molecular models). In spite of this, the extra bulk imposed by the cyclic sulfonamide moiety must be such as to prevent effective binding to GABA receptor sites.

However, quite unexpectedly, compound **7** (TAG) has all the properties of a selective antagonist of taurine.



taurine

Compounds **8** and **9** were also found to be antagonists of taurine but were not studied in detail. Thus, iontophoretic application of **7** (25–100 nA for 30–300 s) readily and reversibly antagonized the inhibitory effects of taurine but not those elicited by GABA on six of six Purkinje cells tested. This selective antagonism on one cell is exemplified in Figure 1, which again illustrates the above-described lack of interaction with GABA systems. Furthermore, the depolarizing actions of taurine, but not of GABA or glycine, on the dorsal root fibers of the isolated amphibian spinal cord were also blocked by compound **7**.[13] These data, as well as a substantially larger body of information involving other experimental preparations to be reported elsewhere,[13] demonstrate that compound **7** represents a totally novel pharmacological agent. Thus, it appears to represent the first selective antagonist of taurine-preferring receptors in the mammalian CNS.

The distance between the acidic and basic groups of taurine is one atom less than that found in **7**, but if the aminomethyl group is rotated toward C-5, the through-space distance does correspond closely to the distance between the amino and sulfonic acid groups of taurine. At the same time, the taurine receptor may recognize the sulfonamide unit in **7** as part of the structure of taurine,

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which would thus contribute to its binding to the receptor, but further work will be required to fully identify the structural features responsible for its antagonistic properties. Nonetheless, the availability of a specific antagonist of taurine may be of considerable utility in helping to delineate the various functions of this relatively ubiquitous amino acid.[14]

Experimental Section

Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Varian Associates EM-360 (60 MHz) and EM-390 (90 MHz) instruments, and chemical shifts are reported in parts per million (δ) relative to Me_4Si or $Me_3SiCD_2CD_2CO_2Na$ as internal standards. The spectra of all compounds in this work were straightforward and unambiguous; hence, only that of the key compound **7** is reported. IR spectra were determined on a Perkin-Elmer 257 spectrophotometer, and UV spectra were taken with a Cary 210 instrument. Mass spectral analyses were provided by Morgan Schaffer Corp., Montreal, and elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN.

6-Bromo- (3) and 8-Bromo-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (4). To 500 g of polyphosphoric acid, stirred and heated at 180 °C, was added 50 g (0.18 mol) of a mixture of **1** and **2**[11] and 100 mL of acetic acid. During the 1st h a vigorous evolution of CO_2 occurred and after 3 h the reaction was complete (TLC). The reaction mixture was cooled to 100 °C and poured onto 1.5 kg of ice. The brown precipitate was filtered, washed well with water, and dried to yield 35 g of crude product, enriched in **3**. This was dissolved in 1 L of acetone, stirred with 3 g of charcoal for 18 h, filtered, and evaporated. The residue was slurried with 100 mL of EtOAc, and the resulting solid was filtered to give 27.5 g of material satisfactory for the following step. From the original aqueous filtrate by extraction with EtOAc there was obtained a further 5.3 g, enriched in **4**: total yield 32.8 g (66%).

A portion of the product was chromatographed over E. Merck silica gel using 10% MeOH in EtOAc as eluent to obtain the individual isomers **3** and **4**. **3**: mp 312–316 °C (lit.[15] mp 309–310 °C). Anal. ($C_8H_7BrN_2O_2S$) C, H, Br, N, S. **4**: mp 260–262 °C. Anal. ($C_8H_7BrN_2O_2S$) C, H, Br, N, S.

6-Cyano- (5) and 8-Cyano-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide (6). A mixture of 31.5 g (0.115 mol) of **3** and **4**, 45 g (0.51 mol) of CuCN, and 250 mL of DMF was heated to reflux for 18 h. The mixture was then poured into 1.5 L of ice-cold water and extracted with 3 × 500 mL of EtOAc. After washing, drying, and evaporating the organic extracts, there was obtained 17 g (67%) of crude **5** and **6**. The isomers were separated by chromatography over E. Merck silica gel using 10% MeOH in EtOAc as eluent, **5** eluting well ahead of **6**. **5**: mp 328–331 °C. Anal. ($C_9H_7N_3O_2S$) C, H, N, S. **6**: mp >350 °C. Anal. ($C_9H_7N_3O_2S$) C, H, N, S.

6-(Aminomethyl)-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Hydrochloride (7·HCl). A mixture of 4 g (18 mmol) of **5**, 280 mL of EtOH, 10 mL of DMF, 10 mL of $CHCl_3$[12] and 500 mg of PtO_2 was shaken on a Parr hydrogenator under 50 psi of hydrogen at room temperature for 20 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated to dryness to give 3.65 g (77%) of product containing small amounts of less polar impurities. This was refluxed three times with 15 mL of MeOH, which dissolved the impurities, to give 1.77 g (37%) of **7**·HCl as a snow-white powder: mp 334–336 °C dec; ^{1}H NMR (D_2O) δ 2.50 (3 H, s, CH_3), 4.40 (2 H, s, CH_2), 7.47 (1 H, d, H_5, $J_{H_5-H_7}$ = 1.5 Hz), 7.60 (1 H, dd, H_7, $J_{H_7-H_5}$ = 1.5 Hz, $J_{H_7-H_8}$ = 8.4 Hz), 7.93 (1 H, d, H_8, $J_{H_8-H_7}$ = 8.4 Hz); UV (0.1 N HCl) λ_{max} 214 nm (ϵ 25 500), 264 (8680). Anal. ($C_9H_{11}N_3O_2S$·HCl) C, H, Cl, N, S. Note: Two difficulties were occasionally encountered in the preparation of **7**·HCl. In some runs it was necessary to add fresh catalyst after 3–4 h to bring the reduction to completion, and in some cases the product crystallized extensively from the hydrogenation mixture. In this case it was easily recovered by slurrying the catalyst and Celite with water, in which the product is very soluble.

8-(Aminomethyl)-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Hydrochloride (8·HCl). Under conditions similar to those used to prepare **7**·HCl, using 1.4 g of **6** as starting material, there was obtained 1.02 g (61%) of **8** as its hydrochloride hemihydrate, mp 328–330 °C dec. Anal. ($C_9H_{11}N_3O_2S$·HCl·0.5H_2O) C, H, Cl, N, S.

6-[(Dimethylamino)methyl]-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-Dioxide Hydrochloride (9·HCl). A solution 3.0 g (11.5 mmol) of **7**·HCl in a mixture of 31 mL of formic acid and 27 mL of 37% aqueous formaldehyde was heated to reflux for 2 h. The reaction was then evaporated to dryness, and the residue was partitioned between 100 mL of 1 N $NaHCO_3$ and 200 mL of warm EtOAc. The aqueous layer was further extracted with 2 × 100 mL of EtOAc, and the combined organic extracts were washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated to leave 1.94 g (67%) of crude solid product. This was suspended in 30 mL of MeOH, and gaseous HCl was passed through until a solution was obtained. The solvent was evaporated, the residue was dissolved in 40 mL of water, and the solution was heated at 90 °C for 1 h with charcoal to remove impurities. The water was evaporated, and the charcoal treatment was repeated three times, which resulted in complete removal of impurities (TLC). The resulting solid salt was slurried with 5 mL of MeOH, filtered, and dried to give 1.25 g (37%) as its hydrochloride hemihydrate, mp 300–302 °C. Anal. ($C_{11}H_{15}N_3O_2S$·HCl·0.5H_2O) C, H, Cl, N, S.

6-Bromo- (10) and 8-Bromo-2-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-Dioxide (11). Into a suspension of 42 g (0.152 mol) of a mixture of **1** and **2**[11] in 300 mL of MeOH was passed gaseous ammonia until a solution was obtained. The solvent was evaporated, and the residue was slurried with 50 mL of MeOH and filtered to give 38 g of the ammonium salt. This was dissolved in 200 mL of DMF, and 20 g (0.141 mol) of methyl iodide was added. After 18 h at room temperature, the reaction was complete (TLC). The mixture was poured into 1 L of water and extracted with 3 × 300 mL of EtOAc. From the organic extracts there was obtained, after drying and evaporation, 40 g of crude, dark product. The isomers were separated by chromatography over E. Merck silica gel using 5% THF in EtOAc as eluent. The separation was difficult and had to be repeated three times on center fractions to give 18.2 g (41%) of **10** and 5.66 g (13%) of **11**. **10**: mp 230–232 °C. Anal. ($C_8H_7BrN_2O_3S$) C, H, Br, N, S. **11**: mp 230–240 °C. Anal. ($C_8H_7BrN_2O_3S$) C, H, Br, N, S.

6-Cyano-2-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-Dioxide (12). Under the same conditions as used to prepare **5** and **6**, from 14 g of **10** there was obtained 8.65 g (76%) of **12**, mp 225–227 °C. Anal. ($C_9H_7N_3O_2S$), C, H, N, S.

8-Cyano-2-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-Dioxide (13). Using the same conditions as for the preparation of **5** and **6**, from 5 g of **11** there was obtained 2.85 g (70%) of **13**, mp 248–250 °C. Anal. ($C_9H_7N_3O_2S$) C, H, N, S.

6-(Aminomethyl)-2-methyl-2*H*-1,2,4-benzothiadiazin-3-(4*H*)-one 1,1-Dioxide Hydrochloride (14·HCl). Hydrogenation of 6 g of **12** under the same conditions as used to prepare **7**, extraction of the crude product into 200 mL of water, and evaporation gave ~7 g of crude hydrochloride. This was redissolved in 60 mL of water and filtered to remove the bulk of some insoluble impurities. The filtrate was heated at 90 °C for 2 h with charcoal, filtered, and evaporated. The residual solid was triturated with 15 mL of MeOH, filtered, and dried to yield 3.7 g (53%) of **14**·HCl, mp 290–300 °C dec. Anal. ($C_9H_{11}N_3O_3S$·HCl) C, H, Cl, N, S.

8-(Aminomethyl)-2-methyl-2*H*-1,2,4-benzothiadiazin-3-(4*H*)-one 1,1-Dioxide (15). A solution of 2 g of **13** in 150 mL of EtOAc, 150 mL of MeOH, and 20 mL of $CHCl_3$ was hydrogenated over 2 g of PtO_2 for 6 h. The reaction mixture was filtered, and the filtrate was evaporated to leave the crude hydrochloride. This was suspended in 60 mL of MeOH and treated with a slight excess of triethylamine. The resulting solution was evaporated, and the solid residue was slurried with 10 mL of MeOH and

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filtered, yielding 1.12 g (55%) of the free base **15**, mp 190–192 °C. Anal. ($C_9H_{11}N_3O_3S$) C, H, N, S.

6-[(Dimethylamino)methyl]-2-methyl-2*H*-1,2,4-benzothiadiazin-3(4*H*)-one 1,1-Dioxide Hydrochloride (16·HCl). A solution of 1.8 g (6.47 mmol) of 14·HCl in 20 mL of formic acid and 18 mL of 37% aqueous formaldehyde was heated to reflux for 2 h. The reaction was then evaporated to dryness, 100 mL of 1 N Na_2CO_3 was added to the residue, and the mixture was extracted with 3 × 100 mL of EtOAc. The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated to leave 1.42 g of crude solid product. This was dissolved in 50 mL of MeOH, and a slight excess of dry HCl gas was passed into the solution, which was then evaporated to dryness. The solid residue was triturated with 10 mL of MeOH, and the solid was filtered and dried to give 1.32 g (67%) of 16·HCl, mp 256–260 °C dec. Anal. ($C_{11}H_{15}N_3O_3S$·HCl) C, H, Cl, N, S.

Biological Methodology. Preparation of Synaptosomes. A crude synaptosomal preparation (P_2) was prepared at 4 °C by homogenizing whole rat brain minus cerebellum in 10 volumes of 0.32 M sucrose with eight strokes of a motor-driven (500 rpm) Teflon–glass homogenizer (Type B, A.H. Thomas, Philadelphia, PA). The resultant homogenate was centrifuged at 1000*g* for 10 min, and the supernatant was decanted and centrifuged at 27000*g* for 20 min. The final P_2 pellet was then used to study GABA synaptosomal processes.

GABA Uptake and Transaminase. GABA uptake and transaminase in rat synaptosomes were measured simultaneously. Synaptosomes were incubated in the presence of 100 μM test compound and [^{3}H]GABA (25 μM) in Krebs–Ringer phosphate buffer for 10 min at room temperature. The reaction was terminated by the addition of 1 mL of a solution of 1 mM unlabeled GABA and 1 mM succinic acid. The reaction tubes were then centrifuged at 1500*g* for 3 min, and 1 mL of the resultant supernatant was transferred to a Dowex 50 column. The [^{3}H]-succinate was eluted with 3 mL of water, and the combined effluent was mixed with 10 mL of Econofluor scintillation cocktail (New England Nuclear, Boston, MA) and counted by liquid scintillation spectroscopy.

The pellet remaining was washed twice with saline, and 0.5 mL of Protosol (NEN) was added to solubilize the tissue. An aliquot (0.25 mL) of the solubilized tissue was added to a scintillation vial, and 10 mL of Econofluor scintillation cocktail was added to determine residual radioactivity. Activity was expressed as a percent of control where this parameter = (counts per minute of drug/counts per minute of control) × 100. Control tissue contained no drug.

GABA Release. GABA release from rat forebrain synaptosomes was measured by a modification of the method of Cotman et al.,[16] the [^{3}H]GABA released being isolated by centrifugation using the microfuge (Beckman Instruments).

GABA Receptor Binding. Interaction of the test compounds with rat brain synaptosomal GABA receptors was measured using [^{3}H]muscimol exactly as described by Williams and Risley.[17]

Taurine Antagonism. Conventional single unit recording and iontophoretic techniques in the cerebellar cortex of male, Sprague–Dawley rats (Charles River Laboratories, 240–350 g) were employed. Multibarrel micropipettes, fabricated using W-P instruments, Inc., Omega Dot capillary tubing (tip diameter 5–10 μM), were filled with the following solutions: NaCl (2 M, used for recording and automatic current balancing barrels), taurine TAU, 0.2 M, pH 8.0, Sigma Chemical Co.), GABA (0.2 M, pH 3.8, Aldrich Chemical Co.), and compound **7** (TAG, 0.05 or 0.1 M, pH 7.0–7.2). All compounds, including taurine, were ejected with anodal currents through the use of a Dagan 6400 current pump. The animals were anesthetized with 350 mg/kg ip of chloral hydrate with supplemental injections as required. They were placed in a stereotaxic frame, and body temperature was maintained at 37 °C via a rectal probe and a servo device connected to a heating pad under the animal. After exposure of the cerebellar vermin cortex and placement of the micropipette, 4% agar in 0.9% saline was applied to prevent drying. Purkinje neurons, identified by their characteristic irregular, high-frequency firing and the presence of inactivation potentials were studied.

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Common Anionic Receptor Site Hypothesis: Its Relevance to the Antagonist Action of Naloxone

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Appropriate modification of 14β-methoxy- and 14β-ethoxycodeinone (prepared by alkylation of 14β-hydroxycodeinone) has generated four alkoxy analogues (**3a–d**) of naloxone and naltrexone. These agents were pure narcotic antagonists in contradiction to the predictions of the common anionic receptor site hypothesis, postulated to be of importance in the enhanced antagonism of naloxone. The molecular change from allyl to cyclopropylmethyl on the N atom increased selectivity of these antagonists for the μ receptor to the same extent as found for naloxone. Increase in the length of the C_{14} O-substituent had no effect on receptor selectivity, and ether formation in most cases did not significantly alter oral/parenteral ratios or durations of action.

A number of different hypotheses have been advanced to explain the antagonism conferred on opiates by a variety of *N*-alkyl substituents. The orientation of the N-substituent was shown to be critical for agonist activity in a rigid morphine analogue.[2] In contrast, however, both axially and equatorially confined substituents have been identified in a number of homobenzomorphan agonists.[3] It was suggested that the essential feature of antagonists was that the antagonist substituent was confined to the axial position.[4] Quantum-mechanical calculations of allyl group conformational minima suggested that N-substituent conformation was a key determinant of antagonist activity,[5a,b] while other calculations support the idea that subtle conformational changes in the piperidine ring cause different interactions with the amine binding site.[6]

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