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Short communication

Interaction of thiocolchicoside with [^{3}H]strychnine binding sites in rat spinal cord and brainstem

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Abstract

Radioreceptor binding assays and receptor autoradiography were used to investigate the activity of thiocolchicoside on strychnine-sensitive binding sites in rat brain and spinal cord using [^{3}H]strychnine as a ligand. Thiocolchicoside displaced the binding of [^{3}H]strychnine with an affinity similar to that of unlabeled glycine, and showed a Hill coefficient and proportionality parameter (P) less than unity. The activity of thiocolchicoside toward [^{3}H]strychnine binding sites was confirmed in autoradiographic studies. The results suggest that thiocolchicoside behaves as an allosteric compound acting on the strychnine-sensitive glycine receptor in rat brainstem and spinal cord, and that this interaction may provide a possible mechanism for the myorelaxant activity of this colchicoside derivative, the first clinically useful drug acting on this receptor.

Keywords: Glycine receptor, strychnine-sensitive; [^{3}H]Strychnine binding; Spinal cord, rat; Autoradiography; Thiocolchicoside; Myorelaxant agent

1. Introduction

Thiocolchicoside, a semi-synthetic derivative of naturally occurring colchicoside, has long been used as a myorelaxant in humans. Previous studies aimed at elucidating the molecular mechanism involved in the therapeutic action of thiocolchicoside suggested that this compound exerts its action by interacting with the γ-aminobutyric acid (GABA) receptor (Biziere et al., 1981a,b). Indeed, thiocolchicoside has been shown to inhibit the binding of [^{3}H]GABA to rat cortical (Biziere et al., 1981b) and cerebellar membranes with an IC_{50} value of 2 μM (Biziere et al., 1981a). The selectivity of this interaction has been demonstrated by the lack of activity of thiocolchicoside in radioreceptor binding assays using radiolabeled ligands specific for other neurotransmitter receptors (Biziere et al., 1981b). This GABA agonistic effect has been further confirmed in vivo since thiocolchicoside, but not colchicoside or colchicine, was able to inhibit the tonic seizures induced by picrotoxin (Biziere et al., 1981b). Colchicoside and colchicine were also inactive in displacing [^{3}H]GABA binding from rat cortical membranes (Biziere et al., 1981b).

Of particular interest, however, is the observation that thiocolchicoside does not show any modulatory effect on the binding of [^{3}H]flunitrazepam to brain membranes whereas it displays weaker affinity ($IC_{50} = 15$ μM) for glycine receptors labeled by [^{3}H]strychnine compared to that observed with [^{3}H]GABA and delays the appearance of strychnine-induced seizures (Biziere et al., 1981a).

The strychnine-sensitive glycine receptor is a heteromeric complex, highly concentrated in the caudal region of the brainstem and in the spinal cord. It is formed by α and β subunits whose structure and function have recently been elucidated (Zarbin et al., 1981; Kuhse et al., 1995). It has been demonstrated that the receptor complex contains distinct binding sites for glycine and strychnine, which are allosterically regulated, and a mathematical model has been developed to study the kinetic properties of the interaction between these two sites (Young and Snyder, 1974; Marvizon et al., 1986).

Due to the important role played by this inhibitory neurotransmitter on motor functions and because of the myorelaxant activity of thiocolchicoside, we studied the interaction of this compound with [^{3}H]strychnine binding sites on rat spinal cord membranes and tissue sections.

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2. Materials and methods

2.1. Displacement of [^{3}H]strychnine binding

The synaptosomal membrane fraction from rat spinal cord was prepared by the method of Young and Snyder (1974), stored at −80°C and used within 2 weeks. The binding assay was performed in a final volume of 1.2 ml of 50 mM sodium-potassium phosphate buffer, pH 7.1 containing 2 nM [^{3}H]strychnine (DuPont-New England Nuclear; specific activity 23.5 Ci/mmol), increasing concentrations of either cold strychnine, glycine, or thiocolchicoside, and membranes at a final protein concentration of 0.2–0.4 mg/1.2 ml. The reaction was carried out at 4°C for 10 min and was terminated by rapid filtration through GF-B glass fiber filters. The filters were rapidly rinsed with 5 ml of NaCl 0.15 M. Non-specific binding was determined in the presence of 0.1 mM unlabeled strychnine.

Data were analyzed using the mathematical model of two mutually interacting binding sites previously reported by Marvizon et al. (1986).

2.2. [^{3}H]Strychnine autoradiography

Autoradiographic detection of the binding sites labeled by [^{3}H]strychnine was performed as described by Bristow et al. (1986). Spinal cord and brain parasagittal sections (20 μm), from male adult Sprague-Dawley rats, were thaw mounted on gelatine-coated slides and stored at −20°C for no more than 2 weeks.

On the day of use, the slide-mounted sections were brought to room temperature and preincubated for 45 min in 50 mM sodium-potassium phosphate buffer, pH 7.1, at 20°C to remove endogenous ligand from the binding sites. Sections were then incubated for 20 min at 4°C in the same buffer containing 8 nM [^{3}H]strychnine. Non-specific binding was determined in the presence of 1 mM of either strychnine, glycine or thiocolchicoside. The incubation was terminated by a rapid dip wash in cold sodium-potassium phosphate buffer, pH 7.1, followed by a 2-min wash in the same buffer and a dip wash in cold water. Autoradiographic images were generated by placing ^{3}H-sensitive film (Amersham) in contact with the slides for 8–10 weeks. Metacrilate ^{3}H-microscale standards (Amersham) were exposed with the sections to allow for quantitative analysis of the autoradiograms, using a computerized image analyzer and the 'Image' software developed by Wayne Rasband (N.I.H.).

3. Results

The ability of thiocolchicoside to displace [^{3}H]strychnine binding from spinal cord membranes was compared with that of strychnine and glycine. Strychnine itself was found to be the most potent compound whereas the potency of thiocolchicoside was similar to that of glycine and, like glycine, the colchicoside derivative was unable to inhibit completely [^{3}H]strychnine binding (Fig. 1). The quantitative evaluation of the displacement curves further confirmed this observation. Thus, the IC_{50} value for strychnine (17.5 ± 2.9 nM) was much lower than that found for glycine and thiocolchicoside (2510 ± 62 nM and 1589 ± 31 nM, respectively). Furthermore, the Hill coefficient (n_H) calculated from displacement curves was less than unity for glycine and was even smaller for thiocolchicoside ($n_H = 0.72 \pm 0.06$ and $n_H = 0.43 \pm 0.08$ for glycine and thiocolchicoside, respectively). These data raise the possibility that thiocolchicoside, like glycine, behaves as an allosteric modulator interacting with a site on the receptor molecule different from that of the radiolabeled ligand.

The properties of this kind of interaction are described by a mathematical model of two mutually interacting binding sites previously reported for the stychnine-sensitive glycine receptor. Thus, the binding data were analyzed using the partial inhibition model to obtain the P (partiality) parameter which indicates the strength of the allosteric inhibitory interaction between the ligand and the inhibitor-binding sites on the receptor molecule. The value of P is between 0 and 1; as P approaches unity, the inhibitory effect becomes smaller. The low values of P obtained in our binding assay ($P = 0.44 \pm 0.08$ and $P = 0.25 \pm 0.03$ for glycine and thiocolchicoside, respectively) suggest that this compound, like glycine, exerts a potent inhibitory allosteric effect on the binding of [^{3}H]strychnine.

In order to ascertain whether the displacement of [^{3}H]strychnine was restricted to a selective region of the

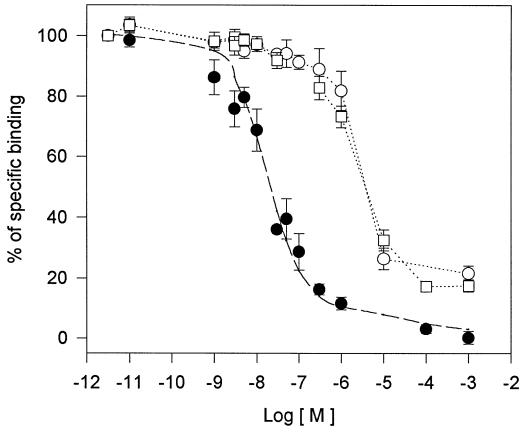

Fig. 1. Displacement of [^{3}H]strychnine binding by strychnine, glycine and thiocolchicoside. Rat spinal cord membranes were incubated with 2 nM [^{3}H]strychnine in 50 mM sodium-potassium buffer, pH 7.1, at 4°C, in the presence of increasing concentrations of strychnine (closed circles), glycine (open circles) and thiocolchicoside (open squares). Sigmoidal curve-fitting was generated by an iterative computer program using the mathematical model described by Marvizon et al. (1986). The data are the means ± S.E.M. of three independent experiments performed on different days and with different membrane preparations.

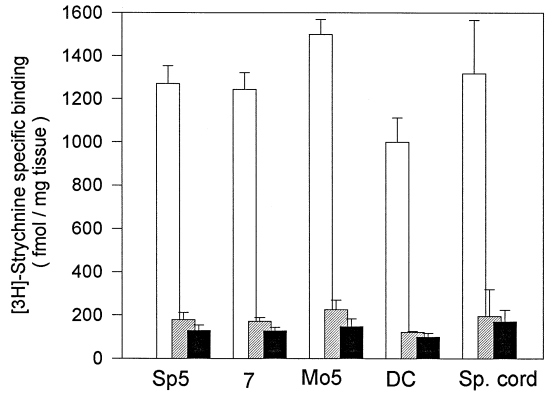

Fig. 2. Quantitative evaluation of [^{3}H]strychnine binding performed with rat brain and spinal cord sections and its displacement by glycine and thiocolchicoside. Parasagittal rat brain and coronal spinal cord sections were incubated with 8 nM [^{3}H]strychnine in sodium-potassium phosphate buffer, pH 7.1, at 4°C, in the absence (open bars) and presence of either unlabeled strychnine, glycine (hatched bars) and thiocolchicoside (closed bars) at 1 mM concentration. Non-specific binding was considered the signal obtained in the presence of unlabeled strychnine. For quantitative evaluation of specific signals, the sections were exposed for 10 weeks together with ^{3}H-microscale standards. Data are the means ± S.E.M. from autoradiograms obtained from three different animals processed in the same experiment. Abbreviations: Sp5, nuclei spinal tract trigeminal nerve; 7, facial nuclei; Mo5, motor trigeminal nuclei; DC, dorsal choclear nuclei.

spinal cord or to specific nuclei of the brainstem, we evaluated the effect of 1 mM unlabeled glycine and thiocolchicoside on [^{3}H]strychnine binding in spinal cord and parasagittal rat brain sections.

As previously reported, the distribution of the radioligand was rather homogeneous throughout the spinal cord sections whereas in the brainstem, the density of binding sites showed a descrete distribution, with highest levels of [^{3}H]strychnine binding found in specific nuclei such as motor trigeminal nuclei, facial nuclei, nuclei spinal tract trigeminal nerve and cochlear nuclei. Moderate binding signals were found in the superior and inferior colliculus, substantia nigra pars reticulata and amygdala. When the sections of either spinal cord or brainstem were incubated in the presence of unlabeled glycine or thiocolchicoside, the specific binding of [^{3}H]strychnine, as revealed by quantitative analysis, was displaced by 80–90% (Fig. 2), confirming the results obtained with binding assays performed with membranes.

4. Discussion

The results presented here show the interaction of thiocolchicoside with strychnine-sensitive glycine receptors. Evidence has been presented suggesting that the binding of the colchicoside derivative occurs at the glycine binding site of these receptors. Thus, in displacement binding studies, thiocolchicoside and glycine displayed similar affinities for [^{3}H]strychnine binding on spinal cord membranes and abolished the autoradiographic signal in the brainstem and spinal cord. The interaction appears to be specific for this kind of receptor since thiocolchicoside could not substitute for glycine in the activation of *N*-methyl-D-aspartic acid (NMDA) receptors labeled by tritiated 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK801) (data not shown).

It has been suggested that thiocolchicoside could exert its muscle relaxant effect by acting on the GABAergic system since it displaces [^{3}H]GABA in binding assays performed with brain membranes and antagonizes the tonic seizures induced by picrotoxin administration in mice (Biziere et al., 1981b). It must be noted, however, that picrotoxin might also act as a potent glycine receptor antagonist (Taleb and Betz, 1994), at least in *Xenopus* oocytes expressing the human glycine receptor α_1 subunit. Moreover, the binding of radiolabeled benzodiazepines to the same receptor complex was not affected by thiocolchicoside (Biziere et al., 1981a) and a weak effect on the binding of [^{3}H]strychnine and a delay in the onset of strychnine-induced seizures by thiocolchicoside were also shown in the same report.

The finding that thiocolchicoside also affected [^{3}H]strychnine binding, however, was underestimated because the study was performed on brain regions which express low levels of receptors (cortex and cerebellum), whereas it has been documented that this type of receptor is mainly localized in the brainstem and spinal cord of rats and mice (Frostholm and Rotter, 1985; Bristow et al., 1986). Indeed, using spinal cord membrane preparations we found that the potency of thiocolchicoside to displace [^{3}H]strychnine binding (IC_{50} = 1.58 μM) is comparable to that reported for the displacement of [^{3}H]GABA (IC_{50} = 2 μM). Whether this similar affinity of thiocolchicoside for both receptors may account for its pharmacological activity remains to be established.

It has been reported, however, that besides its myorelaxant property thiocolchicoside shows analgesic and central nervous system depressant activities (Janbroers, 1987). The activation of GABA receptors by thiocolchicoside might explain, at least in part, its weak central depressant effect, whereas the muscle relaxant activity could be mediated by its interaction with glycine receptors located in the spinal cord. Glycine and glycine receptors, in fact, are remarkably abundant in all layers and all segments of the spinal cord. In particular, at a cellular level, the neurotransmitter is highly concentrated in interneurons but absent in motoneurons which, however, express high levels of receptors (Van den Pol and Gorcs, 1988). This suggests that activation of glycine receptors located on motoneurons inhibits the functional activity of these cells, implying that the myorelaxant activity of thiocolchicoside could be mediated, at least in part, by its agonistic interaction with spinal strychnine-sensitive glycine receptors.

Finally it must be noted that thiocolchicoside seems to be the first drug, already in clinical use, showing affinity for glycine receptors in spinal cord and brainstem.

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