IDENTIFICATION AND CHARACTERIZATION OF PIPECOLIC ACID BINDING SITES IN MOUSE BRAIN

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Pipecolic acid (PA, piperidine-2-carboxylic acid) is the major product of lysine metabolism in the mammalian brain (Giacobini et al., 1980). In this study we have characterized the binding of [3H]PA to P2 fraction membranes and its distribution in the mouse brain. The binding was found to be saturable (70 nM), temperature and Na+ and Cl− dependent. A high affinity binding site with an apparent KD of 33.2 nM and a Bmax of 0.2 pmol/mg protein was demonstrated. The regional distribution of [3H]PA specific binding in mouse brain showed the highest concentration in cerebral cortex, thalamus and olfactory bulb. Unlabeled PA (10⁻³–10⁻¹¹ M) displaced specific binding of [3H]PA in a concentration dependent manner. Out of several substances tested, only proline showed a similar pattern of displacement. Pre-incubation of the membrane preparation with GABA (10⁻³–10⁻¹¹ M) resulted in either an increase or decrease of [3H]PA binding depending on the concentrations of GABA and PA. These results suggest a modulatory action of GABA on PA binding sites. The postnatal development of [3H]PA specific binding was studied in the whole brain of the mouse. [3H]Pipecolic acid binding increased progressively (8-fold) from one day after birth to 16 days. Following this developmental peak, the binding decreased gradually to 30 days at which age, adult values were attained.

INTRODUCTION

Earlier research has pointed out the possible role of several amino acids as synaptic transmitters or modulators (1). More recently, major lysine

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metabolites, such as pipecolic acid (PA) (2,3), L-α-aminoadipic acid (L-α-Aaa) (4) and dicarboxylic acids such as quinolinic acid (5) have been shown to possess neurotransmitter-like characteristics. In the case of PA, the evidence is mainly at the presynaptic level: its regional distribution and its synaptosomal localization (6); its high affinity uptake in synaptosomes (7) and its K+ -induced, Ca2+ dependent release (8). So far, neither characterization of postsynaptic binding sites of PA nor interaction of PA with the membrane receptors of putative transmitters have been reported. This imino acid has been shown to depress the firing of cortical and hippocampal neurons when applied iontophotically (9-10). Intracerebral administration of PA in rat produced sedation, sleeping wave pattern in the electroencephalogram, and other behavioral changes (11). These results suggest the presence of receptor sites for PA in the CNS.

However, the pharmacological effect produced by PA may not necessarily be a result of direct transmitter action, but it may be produced by its interaction with an established neurotransmitter systems. Recent reports of cyclic GABA analogs, especially the six-member ring piperidine-4-sulfonic acid and isonipecotic acid with potent and specific GABA agonist activity (12), suggest the possibility that an alicyclic compound such as PA may act as an endogenous ligand for GABA receptor.

This hypothesis is supported by recent studies in rats, showing that PA or GABA electrophoretically-applied inhibit unit activity of cortical and hippocampal pyramidal neurons (10). Takahama et al. (13) have reported that: (1) PA inhibitory effect was blocked by electrophoretic application of bicuculline but not by strychnine; (2) the inhibitory action of proline and nipecotic acid was little affected by bicuculline; (3) simultaneous injection of PA with a low current facilitated the GABA response, whereas the glycine response was only slightly facilitated by PA; and, (4) the depressive actions of PA, and GABA as well, were not affected in Ca2+ -free, high Mg2+ medium. These results suggest a relationship between the postsynaptic action of PA and GABA receptors.

In this study a high affinity binding site for PA has been characterized in mouse brain and its possible relationship with the GABA system has been investigated. A preliminary report of this study was published by Giacobini and Gutierrez (14).

**EXPERIMENTAL PROCEDURE**

Reagents. [3H]-Pipecolic acid hydrochloride ([3H]PA (2.98 Ci/mmol) obtained by custom tritium labeling (New England Nuclear Corp., Boston, MA) was routinely monitored and purified if necessary as described below. D,L-Pipecolic acid, L-proline, α-Aaa, glutamate, aspartate, glycine and piperidine were from Sigma Chemicals (St. Louis, MO). The GABA