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Does Pipecolic Acid Interact with the Central GABA-ergic System?

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With 4 Figures

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Summary

Several previous studies have suggested a strong GABA-mimetic action of the endogenous brain imino acid, L-pipecolic acid (L-PA). In the present study, these observations were evaluated using electrophysiological and neurochemical methods. In contrast to published data our electrophysiological studies on rat cortical neurones *in situ* showed only a weak, but bicuculline-sensitive depressant action of L-PA on cortical neurones.

Furthermore, L-PA proved to have no affinity for any of the three components of the GABA-benzodiazepine-chloride channel receptor complex. However, using a modification of published methods a weak affinity for the GABA-B receptor site was demonstrated ($IC_{50} = 1.8 \times 10^{-3}$ M). L-PA showed no anticonvulsive activity in several tests; in particular, it did not protect mice from seizures induced by inhibition of L-glutamate-1-decarboxylase (EC 4.1.1.15: GAD). L-PA had a very weak action on brain GABA levels of mice, and did not modify the rate of GABA synthesis. In conclusion, these results are not compatible with a strong *in vivo* interaction between L-PA and GABA-mediated inhibitory transmission.

Key words: Pipecolic acid, GABA, Micro-iontophoresis, GABA-B receptors.

Introduction

Pipecolic acid (2-piperidinocarboxylic acid, PA), is a major metabolite of lysine and a normal constituent of the mammalian brain

(Kase *et al.*, 1973). While lysine is mainly metabolized via saccharopine in most organs, much evidence has been accumulated recently suggesting that conversion to PA may be the major metabolic pathway of lysine in brain (Chang, 1976; 1978 a, b; 1982; see also Giacobini 1983; and Giacobini and Gutierrez 1983 for a review). Giacobini and co-workers have demonstrated a saturable, temperature- and Na^+-dependent uptake of PA in brain synaptosomes, a Ca^{++}-dependent release from brain slices, a saturable transport across the blood-brain barrier (BBB), and a high-affinity, Na^+-dependent specific binding of [^{3}H]-PA in the mouse CNS ($K_D = 33.2$ nM) that is associated with the GABA system (Giacobini *et al.*, 1980; Giacobini and Gutierrez, 1983; Gutierrez and Giacobini, 1985).

PA has been shown to increase GABA release from brain slices (Okuma *et al.*, 1979; Nomura *et al.*, 1980) and to decrease its uptake in synaptosomes (Nomura *et al.*, 1978). GABA uptake into glia is also inhibited by PA (Nomura *et al.*, 1981). Since this uptake is one of the major mechanisms for regulating the concentration of GABA in the synaptic cleft, these results suggest that PA may potentiate the action of GABA in the CNS by increasing its level in the cleft. Such a hypothesis is supported by electrophysiological results, which have demonstrated a bicuculline-sensitive inhibitory effect of PA applied by iontophoresis to neocortical and hippocampal neurones (Kase *et al.*, 1980; Takahama *et al.*, 1982).

The interaction between PA and GABA seems to be specific for this imino acid, since no such effects were found with other neurotransmitters such as NA, 5-HT, DA and glycine (Nomura *et al.*, 1978; Okuma *et al.*, 1979). However, an action of PA on the GABA system was only observed at the very high concentration of 10^{-4} M. Since PA is present in relatively low concentrations in mouse brain (less than 10 nmol/g), the effects described above may be physiologically important only if PA is highly concentrated in some brain areas. Recently, Kim and Giacobini (1984) reported a regional distribution of L-PA in the mouse brain, with values varying from 8.13 ± 1.14 nmol/g tissue in the cerebellum to 2.58 ± 0.28 nmol/g tissue in the spinal cord. However, the ratio of 3.2 of these concentrations seems too low to explain the discrepancies between the potent electrophysiological effects reported by the groups of Kase and Takahama and published results on the action of L-PA on the GABA transport system.

The aim of this study was to investigate the effect of PA in several electrophysiological, pharmacological and biochemical test systems, some of which are known to involve GABA as a neurotransmitter, in

order to examine whether or not an interaction between PA and this inhibitory neurotransmitter may occur in the brain.

Materials and Methods

Animals

The effects of L-PA on GABA levels, GABA turnover, protection against convulsions and *in vivo* ^{3}H-flunitrazepam binding were investigated in male mice (Tif: MAGf, SPF), aged 5–8 weeks and weighing 23–27 g. Electrophysiological experiments were performed on male albino rats (Tif: RAIf, 250–350 g). For *in vitro* binding assays, male rats (Tif: RAIf, 200 g) were used. The animals were housed six to a cage and allowed water and food *ad libitum*. The mice and the rats were kept in an air-conditioned room at 21–22 °C, with a 12-hour light-dark cycle. In each biochemical experiment the animals were sacrificed between 8.30 and 10.00 a.m. to avoid possible circadian variations in the endogenous levels of neuro-active amino acids.

Chemicals

GABA and pentafluoropropionic acid were purchased from Fluka AG (Buchs, Switzerland). Hexafluoropropanol was from Merck (Darmstadt, Germany). Gabaculine hydrochloride and β-vinyllactic acid were synthesized in our laboratories by Dr. W. Bencze. [^{3}H] Muscimol (specific activity 31.0 Ci/mmol), [^{3}H] flunitrazepam (specific activity 77.4 Ci/mmol) and [^{35}S]t-butyl-bicyclophosphorothionate (TBPS, specific activity 65.0 Ci/mmol) were obtained from New England Nuclear; [^{3}H] baclofen was synthesized at CIBA-GEIGY Horsham (specific activity 7.5 Ci/mmol). PA was obtained from several suppliers (Fluka, Sigma, EGA). The different lots were more than 99 % pure as judged from thin-layer chromatography.

Electrophysiological Experiments

Micro-iontophoretic Studies

The rats were anaesthetized with chloral hydrate (400 mg/kg i.p.) and additional anaesthetic (100 mg/kg) was given when required. Body temperature was maintained at 37 ± 0.5 °C. Conventional electrophysiological techniques were used to record neuronal activity extracellularly and to apply substances by iontophoresis. The neurones studied were spontaneously active cells located between 600 and 1500 μ from the pial surface in the anterior cingulate cortex or the parietal cortex. Recordings were made via one barrel of 3- or 4-barrelled glass micropipettes, which was filled with NaCl (4 M) to balance the iontophoretic currents. Action potentials from single cells were amplified and displayed on an oscilloscope screen. They were counted by means of a variable voltage gate and ratemeter and integrated over periods of 4–10 s. The histogram outputs of cell firing rate were displayed on a chart recorder. The remaining barrels of the pipette contained

solutions for iontophoretic application of the substances. The following substances were used: D, L-PA (EGA, 0.5 M, pH 6–7, dissolved in NaCl 0.165 M); L-PA (Sigma, pH 6–7 or pH 4, dissolved in NaCl 0.165 M); D-PA (Fluka, as for D, L-PA); GABA (Serva, 0.1 M, pH 4.0); bicuculline (Sigma, 0.001 M, pH 5.0).

Receptor Assays

^{3}H-flunitrazepam Radioreceptor Assay *in vitro* and *in vivo*

The *in vitro* assay was a modification of that described by Speth *et al.* (1980) and published by Dooley and Bittiger (1982). The assay *in vivo* was based on the method previously published by Chang and Snyder (1978) with minor modification.

GABA-A Radioreceptor Assay

This assay was a modification of that described by Beaumont *et al.* (1978).

Preparation of membranes: Rats were decapitated and the cerebral cortices dissected and homogenized in 6 volumes of sucrose (0.32 M, containing 1 mM K_2HPO_4 and 1 mM $MgCl_2$) in a glass-Teflon homogenizer. The suspension was sedimented (1000 × g for 10 min) and the resulting supernatant was centrifuged (8000 × g for 15 min at 4 °C). The pellets were resuspended in Tris-HCl buffer (1 mM, pH 7, containing 2 mM EDTA) and left for 40 min at 4 °C. The material was centrifuged twice (30,000 × g for 20 min) and the pellets suspended in Tris-citrate buffer (50 mM, pH 7.1 at 20 °C).

Radioreceptor assay: Membranes (about 300–500 μg protein) were incubated (7 min at room temperature) in 1 ml Tris-citrate buffer (50 mM, pH 7.1) containing ^{3}H-muscimol (1 nM) and L-PA or L-proline and then centrifuged for 8 min at 12,000 × g. The binding observed in the presence of high concentrations of GABA (10^{-4} M) was termed non-specific. The supernatants were aspirated and the tips of the tubes containing the pellets were cut off and transferred to counting vials containing 1 ml Soluene (Packard). After dissolution of the pellets, 10 ml of acidified Irgascint (Ciba-Geigy) was added and the samples counted in an Intertechnique liquid scintillation counter at an efficiency of 42 %.

GABA-B Radioreceptor Assay

The method used was as described previously (Hill and Bowery, 1981) with some modifications. By using cerebellar tissue and including a density gradient step, specific binding of ^{3}H-baclofen could be increased.

Preparation of membranes: Rats were decapitated, the brains removed and the cerebella separated. Twenty cerebella were homogenized in 10 volumes of ice cold sucrose (0.32 M containing 1 mM K_2HPO_4, pH 7.2) with a glass-Teflon homogenizer. The homogenate was centrifuged (1000 × g for 10 min)

and the resulting supernatant was recentrifuged (20,000 × g for 15 min). The pellet was resuspended in 10 ml buffer solution. This solution was divided in two 5-ml fractions, which were subjected to a density gradient centrifugation (40,000 × g for 30 min) with one step of 25 ml sucrose (0.8 M, containing 1 mM K_2HPO_4, pH 7.2). The supernatant was aspirated, and the pellet suspended in 25 ml water, kept for 30 min at 4 °C and then centrifuged for 15 min at 40,000 × g. The pellet was covered with a modified Krebs-Henseleit solution (containing 20 mM Tris-HCl, pH 7.4, instead of $NaHCO_3$) and frozen at −20 °C for 2 days. The pellets were then thawed at room temperature, kept suspended for 45 min in Krebs-Henseleit solution and centrifuged (18,000 × g for 10 min). This washing step was repeated 3 times. The pellet was again kept overnight at 4 °C and rewashed 4 times the next day. The final pellet was suspended in 8–10 ml Krebs-Henseleit solution.

Radioreceptor assay: Membranes (about 300 μg protein) were incubated in 1 ml buffer with ^{3}H-baclofen (10 nM) and L-baclofen, L-PA, L-lysine, L-proline or L-4-hydroxyproline for 10 min at 22 °C. The incubation was terminated by centrifugation at 20,000 × g for 5 min. The supernatant was aspirated, the pellet rinsed and the tips of the tubes containing the pellets cut off and transferred in counting vials containing 1 ml of Soluene (Packard). Nonspecific binding was determined in the presence of 10 μM baclofen. Specific binding was more than 60 %. Scintillator was added after dissolution and the radioactivity counted.

[^{35}S]t-Butyl-bicyclophosphorothionate (TBPS) Radioreceptor Assay

TBPS binds to membrane preparations from rat forebrain in a manner suggestive of association with chloride channels (Squires *et al.*, 1983).

Preparation of membranes: Rat forebrains were stored at −80 °C. After thawing, the tissue was homogenized in 50 volumes of Tris-HCl buffer (50 mM, pH 7.3 at 25 °C) using a Brinkman Polytron tissue grinder (setting of 6 for 30 sec). Membranes were sedimented (48,000 × g) for 10 min. Pellets were washed three times in Tris-HCl buffer. After the first resuspension the membranes were incubated for 30 min at 0 °C. After the third wash, the membrane suspension was frozen at −20 °C for at least 16 hours. Immediately before the assay, membrane suspensions were thawed, centrifuged and washed twice as described above. The final pellet was resuspended in 200 volumes (original tissue weight) of Tris-HCl buffer (50 mM, pH 7.3 at 25 °C) containing NaCl (120 mM), KCl (5.0 mM), $CaCl_2$ (2.0 mM) and $MgCl_2$ (1.0 mM).

Radioreceptor assay: A cold membrane suspension (2.0 ml) containing the equivalent of 10 mg fresh brain tissue was added to triplicate test-tubes containing appropriate concentrations of test drugs together with [^{35}S]-TBPS (2.0 nM). The samples were incubated at 21 °C for 90 min, filtered through Whatman GF/B microfilter discs (2.4 cm diameter) and washed three times with 5.0-ml portions of ice-cold Tris-HCl buffer (50 mM, pH 7.3 at 25 °C). Non-specific binding was defined as [^{35}S]-TBPS binding in the presence of 1.0 mM GABA.

Pharmacological Experiments

Electroshock Test, Mouse

Tonic convulsions of the hind extremities were induced by passing alternating electrical current (50 Hz and 18 mA for 0.2 s) through corneal electrodes. PA was administered either perorally one hour or intraperitoneally half an hour before seizure induction. Five animals per dose were used; one group served as control. Carbamazepine, 30 mg/kg p.o. was used as positive control. The number of animals protected from tonic hind-limb extension seizures was determined in each group.

Seizures Induced by Pentetrazole, Picrotoxin or β-Vinyllactic Acid

Clonic seizures were induced in mice by injection of either pentetrazole (70 mg/kg i.p.) or picrotoxin (7.5 mg/kg i.p.). PA was given either perorally one hour or intraperitoneally half an hour prior to the convulsant drugs. Five animals per dose were used; one group served as control. Rivotril, 0.1 mg/kg p.o. was used as positive control. The number of animals protected from clonic seizures was determined in each group. The observation period was 10 min. The published procedure (Bernasconi *et al.*, 1984 b) was used for β-vinyllactic acid-induced seizures.

Determination of GABA Levels and Turnover

Measurement of GABA Level

The methods used for the extraction and derivatization of GABA as well as for the gas-chromatographic determination of the amino acid were as described previously (Schmid and Karobath, 1977; Bernasconi *et al.*, 1982) with minor modifications.

Measurement of GABA Turnover *in vivo* after Inhibition of GABA-T

The method is based on the fact that gabaculine selectively inhibits GABA-T and this inhibition results in a large increase in GABA level. Mice were injected with gabaculine (100 mg/kg i.p.) and sacrificed by microwave irradiation 60 min later. The turnover rate *in vivo* was then estimated from the difference between GABA levels in brains of mice treated with the GABA-T inhibitor and brains of non-treated animals. PA was administered 5 min before gabaculine.

Statistics

Results were expressed as means ± S.E.M. and the statistical significance was determined by Dunnett's *t*-test. For the β-vinyllactic-acid-induced convulsions another statistical method was used: Results were expressed as medians and a trend test according to Jonckheere was carried out (Hollander and Wolfe, 1973).

Results

Electrophysiological Experiments in vivo

The effects of PA, applied by iontophoresis, were tested on 92 cortical neurones, 38 in the anterior cingulate cortex and 54 in the parietal cortex. In the cingulate cortex, 5 cells showed very weak inhibitory effects with iontophoretic currents up to 120 nA. The remaining 33 cells were completely unaffected by PA up to 120 nA. In the parietal cortex, similar, very weak depressant effects were noted, but the proportion of cells affected appeared to be greater (28 out of 54 with the remainder unaffected). Examples of the weak depressant effects of PA on two parietal neurones are shown in Fig. 1. These weak effects contrast greatly with the strong inhibitory effects of GABA on the same neurones. In view of the profound inhibitory actions of PA previously reported by Kase *et al.* (1980), we performed several tests to try to eliminate the possibility that technical factors were involved in the discrepancy between their studies and ours. Thus we used PA from three different sources (Sigma, EGA and Fluka) but found no apparent differences. We also compared the effect of PA applied from solutions at pH 7 and pH 4 on 7 neurones. One cell was unaffected by the imino acid at either pH. The remaining 6 were weakly depressed, but no difference in the degree of depression was noted at either pH. We also considered the possibility that the weak depressant actions of PA may result from ejection of

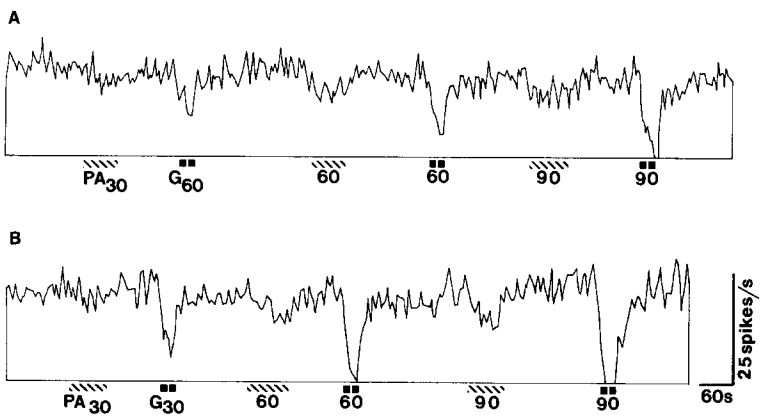

Fig. 1. Examples of records from two parietal cortical cells showing weak depressant effects of PA. The traces are ratemeter records of cell firing. The bars beneath the traces indicate periods of iontophoretic application and the numbers correspond to the intensity of ejecting current in nA. Both cells were strongly depressed by GABA *(G)*, but PA had only weak depressant effects

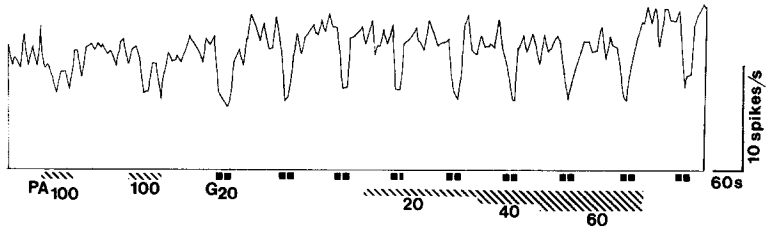

Fig. 2. Interaction between GABA and *PA* on a single parietal cortical cell. Details as in Fig. 1. PA itself had a weak depressant action on this cell. GABA *(G)* was strongly depressant but the depressant effects of G were neither potentiated, nor reduced during the continued application of *PA*, despite a gradual reduction in the baseline firing rate

GABA ions which may have been taken up into the PA-barrel during periods of GABA ejection and PA retention. However, this was considered unlikely, since weak depressant effects could be evoked with PA when the iontophoretic electrode did not contain GABA. We looked for possible interactions between PA and GABA on 8 cells in the parietal cortex. The depressant effects of GABA were neither reduced, nor potentiated during the iontophoretic application of PA on any occasion. One study is illustrated in Fig. 2. PA applied alone to this cell caused weak depression. GABA was strongly depressant, but continuously applied PA, although it gradually reduced the baseline firing rate, did not alter the GABA response. Finally, we examined the ability of bicuculline to block the weak depressant response to PA on 7 parietal cortical cells. On 6 occasions the responses to PA were noticeably reduced during applications of

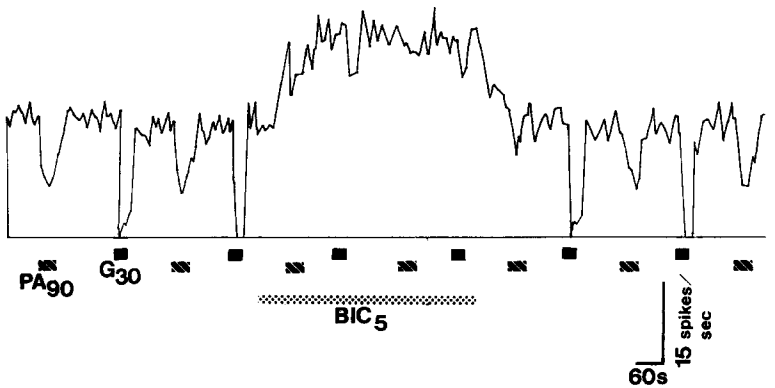

Fig. 3. Blockade of responses of a parietal cortical cell to GABA (G) and PA during the iontophoretic application of bicuculline *(BIC). BIC* application resulted in a strong increase in baseline firing and this was accompanied by abolition of responses to both *G* and *PA*

bicuculline with weak iontophoretic currents (1–5 nA). One study is shown in Fig. 3. The cell was weakly depressed by PA and strongly depressed by GABA. Bicuculline strongly increased baseline firing rate and blocked responses to both agonists. On one further cell, despite an increase in firing rate during the application of bicuculline, the weak depressant responses to PA were not noticeably reduced. Unfortunately, GABA was not tested on this cell.

Binding Data

L-PA showed no affinity for either GABA-A binding sites or for the benzodiazepine recognition sites at concentrations as high as 10^{-3} M. A weak, but reproducible displacement of [^{3}H] baclofen was observed at 10^{-4} M (30 % inhibition, Fig. 4). At 200 mg/kg p.o. no significant change in the binding of [^{3}H] flunitrazepam *in vivo* could be detected. L-PA inhibited [^{35}S]-TBPS binding by less than 10 % at 10^{-4} and 10^{-3} M (Table 1).

Anticonvulsive Tests

L-PA given either perorally (300 mg/kg) or intraperitoneally (200 mg/kg) did not protect mice against electrically induced seizures. No protective effect of PA administered either perorally

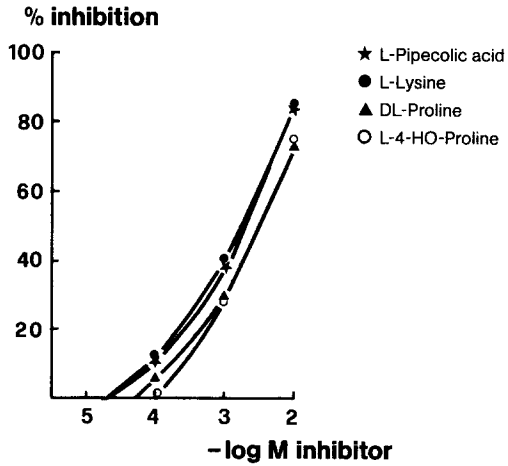

Fig. 4. Competition for [^{3}H]-baclofen specific binding sites by *L-PA, D,L-proline, L-4-hydroxyproline* and *L-lysine*. Specific binding was more than 60 %. The amount bound in the presence of each concentration of unlabelled compound has been plotted as a percentage of this displaceable portion. Each point is the mean of 3 determinations, performed in triplicate. The S.E.M. are smaller than the symbols and are thus not shown

Table 1. *Effects of L-pipecolic acid and analogues in various binding assays*

Ligands	Amino acid	Expression of results
[^{3}H]Flunitrazepam *in vivo*	Pipecolic acid (200 mg/kg p.o.)	20 % decrease of binding
[^{3}H]Flunitrazepam *in vitro*	Pipecolic acid	< 10 % inhibition at 10^{-3} M
	L-Proline	< 10 % inhibition at 10^{-3} M
[^{3}H]Muscimol	Pipecolic acid	< 10 % inhibition at 10^{-3} M
	L-Proline	< 10 % inhibition at 10^{-3} M
[^{3}H]Baclofen	Pipecolic acid	$IC_{50} = 1.8 \times 10^{-3}$ M
	L-Lysine	$IC_{50} = 1.8 \times 10^{-3}$ M
	D, L-Proline	$IC_{50} = 3.5 \times 10^{-3}$ M
	L-4-Hydroxyproline	$IC_{50} = 3.2 \times 10^{-3}$ M
[^{35}S]-TBPS	L-Pipecolic acid	< 10 % inhibition at 10^{-3} M
	L-Proline	< 10 % inhibition at 10^{-3} M

Binding assays were performed in triplicate according to the procedure described in "Materials and Methods".

Table 2. *Pharmacological methods utilized in determining the lack of anticonvulsant activity of pipecolic acid in mice*

Test	Dose of pipecolic acid	Anticonvulsant activity
E-Shock	300 mg/kg p.o.	Ø
	200 mg/kg i.p.	Ø
Pentetrazole	300 mg/kg p.o.	Ø
	600 mg/kg p.o.	Ø
	200 mg/kg i.p.	Ø
Picrotoxin	300 mg/kg p.o.	Ø
	600 mg/kg p.o.	Ø
	200 mg/kg i.p.	Ø
β-Vinyllactic acid	100 mg/kg p.o.	Ø
	600 mg/kg p.o.	Ø
	200 mg/kg i.p.	Ø

The anticonvulsant activity was tested according to the procedures described in "Materials and Methods". Five animals per dose were used except for β-vinyllactic acid where 10 animals per dose were used.

(300 and 600 mg/kg) or intraperitoneally (200 mg/kg) one hour prior to pentetrazole, β-vinyllactic acid or picrotoxin was recorded (Table 2).

Symptomatology

No clear-cut effects could be seen. A slight ataxia was observed in several experiments.

Effect on GABA Level and GABA Turnover

L-PA was administered intraperitoneally at three different doses (50, 150 and 500 mg/kg). The highest dose tested resulted in a slight, but significant ($p < 0.05$) increase in cerebral GABA concentration (to 15 % above control). No change in the rate of synthesis of GABA was seen at the three doses employed (Table 3). Furthermore, the decrease in GABA turnover observed after phenobarbitone (50 and 150 mg/kg p.o.) and diazepam (3, 10, and 30 mg/kg p.o.) was not modified by pretreatment with L-PA (500 mg/kg i.p., results not shown).

Table 3. *Effects of pipecolic acid on gabaculine-induced GABA accumulation in the cortex of mice*

Treatment	GABA (μmol/g wet tissue)
Control	1.30 ± 0.05
Gabaculine (100 mg/kg i.p.)	3.71 ± 0.40***
Pipecolic acid (500 mg/kg i.p.)	1.50 ± 0.04*
Pipecolic acid (50 mg/kg i.p.) + gabaculine (100 mg/kg i.p.)	3.29 ± 0.22 N.S.
Pipecolic acid (150 mg/kg i.p.) + gabaculine (100 mg/kg i.p.)	3.55 ± 0.35 N.S.
Pipecolic acid (500 mg/kg i.p.) + gabaculine (100 mg/kg i.p.)	3.40 ± 0.10 N.S.

Pipecolic acid was administered 65 min prior to sacrifice and gabaculine 5 min after pipecolic acid. The results are the means ± S.E.M. for five mice. Similar results were obtained in the *C. striatum*.

* $p < 0.05$.

*** $p < 0.01$ statistical significance of difference calculated by Dunnett's test.

N.S. When compared to the group treated with gabaculine alone.

Discussion

The effects of iontophoretically applied PA on the activity of cells in the cerebral cortex noted in the present study are in partial agreement with previously reported data (Kase *et al.*, 1980; Takahama *et al.*, 1982). Thus, neurones which were sensitive to the imino acid invariably exhibited a decrease in firing rate. However, whereas the Japanese workers found PA to be an extremely potent depressant agent at low iontophoretic currents (5–15 nA), we found only weak and variable effects at much higher ejecting currents (usually in the 80–120 nA range). Moreover, in the fronto-parietal cortex we found only about 50 % of cells responsive to PA, in contrast to close to 80 % reported by Kase *et al.* (1980). Interestingly, in the cingulate cortex PA was even less effective with only a very small percentage of

cells being weakly affected. The reasons for the differing effectiveness of PA noted in our investigation and that of Kase *et al.* (1980) are unknown, although technical differences could have contributed. Thus, the anaesthesia employed in the two studies was different and also the type of micropipette used. Our pipettes had the recording and iontophoresis barrels at the same level. Kase *et al.* (1980) used electrodes which had the recording barrel cemented separately to and (presumably) with its tip in advance of the iontophoretic pipette. If this was the case then it is possible that PA in their study was applied further out on the dendrites, as opposed to close to the cell bodies in our investigation.

A further difference between our study and those of the groups of Kase and Takahama concerns the interactions between PA and GABA on cortical cells. These authors reported in an abstract (Takahama *et al.*, 1983) that low currents of PA were able to potentiate the depressant actions of GABA on cortical cells. We observed neither potentiation, nor inhibition of GABA-induced depressions during the iontophoretic application of PA.

However, we have confirmed the observation (Takahama *et al.*, 1982) that the depressant actions of PA are sensitive to blockade by the GABA antagonist, bicuculline. On the basis of this finding, it would seem likely that the depressant effects of PA may be the result of an action on the GABA-ergic system (*e.g.* by direct stimulation of GABA receptors, by increased release of GABA, and/or by the inhibition of GABA uptake).

PA showed no affinity even at 10^{-4} M for any of the three components the GABA-benzodiazepine-chloride channel receptor complex. Therefore, it seems more likely that the weak depressant effects of L-PA may result from a release of endogenous GABA and/or a blockade of its re-uptake. The latter effect at least seems unlikely, since L-PA did not potentiate the effects of iontophoretically applied GABA. Whatever the mechanism of the interaction, it would not seem to be pronounced, since the electrophysiological effects were weak and since a potent facilitation of GABA-ergic transmission by PA would result in a reduction in GABA turnover (Bernasconi *et al.*, 1984 a). This conclusion is in keeping with previous studies which have demonstrated that PA blocks GABA uptake and increases GABA release *in vitro* only at high concentrations (Okuma *et al.*, 1979; Nomura *et al.*, 1978, 1980).

The displacement of ^{3}H-baclofen by L-PA, although weak, is interesting. However, this action does not appear to be specific for L-PA, since other imino acids such as D,L-proline, L-4-hydroxyproline and even the amino acid L-lysine, the precursor of L-PA, show the

same affinity for the GABA-B receptor site. Furthermore, some of the pharmacological properties of baclofen are not shared by PA. For instance, in contrast to baclofen (Delini-Stula, 1979), PA does not antagonize seizures induced by the inhibition of GAD.

Moreover, baclofen dose-dependently and stereospecifically reduces the rate of GABA synthesis *in vivo* (Bernasconi and Martin, 1978). No such effect was observed with L-PA, even after such very high doses as 500 mg/kg i.p. of the imino acid. This apparent discrepancy could be related either to poor penetration of PA into the brain or to possible antagonistic, or partial agonistic activity of PA at the GABA-B receptor sites. It would be interesting to determine the effects of i.c.v. administration of PA.

In summary, our results do not support previous findings suggesting that PA possesses strong GABA-mimetic properties. However, a moderate interaction with the GABA-B receptor sites is shown. Micro-iontophoretic studies demonstrate a weak depressant action on cortical neurones which is bicuculline sensitive. This effect may result from the known action of PA on GABA transport systems.

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