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Short communication

The γ-aminobutyric acid uptake inhibitor NO-711 potentiates 3-aminopropylphosphinic acid-induced actions in rat neocortical slices

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Abstract

In rat neocortical slices maintained in Mg^{2+}-free Krebs medium, the $GABA_B$ receptor agonists baclofen and 3-aminopropylphosphinic acid dose-dependently reduced the frequency of spontaneous discharges, 3-aminopropylphosphinic acid being 10 times less potent than baclofen. These were sensitive to the antagonist CGP 52432 (3-[[3,4-dichloro-phenyl)methyl]-amino]propyl](-P-diethoxymethyl)-phosphinic acid) (1, 5 and 10 μM). The GABA uptake inhibitor NO-711 (1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) (5 and 10 μM) produced 2.9 and 9 fold increases in the potency of 3-aminopropylphosphinic acid without affecting baclofen-induced responses. In this study, the low potency of 3-aminopropylphosphinic acid when compared to baclofen, may be attributed to its uptake by NO-711-sensitive GABA transporters. © 1998 Elsevier Science B.V.

Keywords: $GABA_B$ receptor; NO-711; 3-aminopropylphosphinic acid; CGP 52432; Neocortical slice, rat; Uptake inhibitor

1. Introduction

$GABA_B$ receptors were first characterised as bicuculline-insensitive GABA receptors for which baclofen is a selective agonist (Bowery et al., 1980). Subsequently, the phosphinic analogue of GABA, 3-aminopropylphosphinic acid, was found to be a potent displacer of baclofen from $GABA_B$ receptors (Dingwall et al., 1987), and eventually shown to be an agonist for these receptors (Pratt et al., 1989). In peripheral tissues, 3-aminopropylphosphinic acid is generally considered to be more potent as an agonist than GABA or baclofen at $GABA_B$ receptor sites (Hills et al., 1989; Hills and Howson, 1991; Ong et al., 1990a; Chapman et al., 1993), but seems not to be so in a variety of central preparations where it is either equipotent or less potent than baclofen itself (Pratt et al., 1989; Seabrook et al., 1990; Lovinger et al., 1992). Indeed, in the spinal cord, Lacey and Curtis (1994) found that baclofen was some 40 times more potent than 3-aminopropylphosphinic acid in reducing spinal field potentials. In the same way, in spontaneously discharging neocortical slice preparations maintained in Mg^{2+}-free Krebs medium, baclofen depresses the rate of spontaneous activity by activating $GABA_B$ receptors, but 3-aminopropylphosphinic acid at similar concentrations had little or no effect on the discharge rate (Ong et al., 1990b). Field excitatory responses in the hippocampal slice were similarly relatively insensitive to 3-aminopropylphosphinic acid, whereas baclofen effectively inhibits these responses by reducing synaptic transmission (Lovinger et al., 1992). One possible explanation for these anomalies could be that applied 3-aminopropylphosphinic acid may be a substrate for GABA uptake carriers, and thus does not reach effective concentrations at the $GABA_B$ receptor sites. In the present study, we have investigated the pharmacological actions of 3-aminopropylphosphinic acid in the presence of a potent and selective GABA uptake inhibitor NO-711 (1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) (Suzdak et al., 1992), and show a potentiation by NO-711 of the 3-aminopropylphosphinic acid-induced depression of spontaneous discharges in rat neocortical slice preparations. This suggests that the low potency of 3-aminopropylphosphinic acid at $GABA_B$ receptors in the rat neocortex may be attributed to uptake of 3-aminopropylphosphinic acid by GABA transporters.

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2. Materials and methods

2.1. Rat neocortical slice preparations

Outbred male adult Sprague–Dawley rats (250–300 g) were decapitated under halothane anaesthesia, their brains rapidly removed and immersed for 15 min in ice cold Krebs solution oxygenated with 95% O_2 and 5% CO_2. Cerebral cortical slices (400 μm thick) were prepared by cutting coronal sections using a vibraslice microtome (Campden Instruments, UK). Using a superfusion method based on a grease–gap system as previously reported (Ong et al., 1990b), the neocortex was initially superfused with Mg^{2+}-containing Krebs medium at 28°C delivered by a peristaltic pump at 1 ml/min, and allowed to equilibrate for 30 min, before changing to Mg^{2+}-free medium. The composition of the Krebs medium was as follows [mM]: NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.0, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.3, pH 7.4; for the Mg^{2+}-free medium, $MgSO_4$ was omitted. DC potentials between the cingulate cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges, with a high-input impedance DC amplifier, and responses were displayed on a chart recorder.

After a further period of equilibration for 60 min under Mg^{2+}-free conditions, the neocortical slices developed spontaneous paroxysmal discharges. Varying doses of the agonist were then applied to the cortical side of the tissues for 2 min, usually at 30 min intervals, and washed out with recovery of responses to control level. The antagonist was superfused for 2 min prior to, and then continuously with the agonist. The GABA uptake inhibitor NO-711 was superfused over the slice for 15 min before further applications of either the agonist alone, or a combination of the agonist with the antagonist. Data were analysed by counting the number of spontaneous discharges in 5 min epochs, in the absence and presence of test compounds, and the values expressed as a percentage depression of the average control discharge rate measured during the 5 min immediately before the addition of drugs.

Concentration–response curves for each agonist were constructed in the presence and absence of the antagonist, and then repeated in the combined presence of the GABA uptake inhibitor NO-711. As a measure of agonist potency, the half-maximally effective concentration (EC_{50}) was the concentration of the agonist required to produce 50% depression of spontaneous activity, estimated from the concentration–response curve. For antagonist potency, the pA_2 value was determined using the relationship $pA_2 = \log(CR-1)\text{-}\log[B]$, where $(CR-1)$ is the concentration ratio -1, and [B] the antagonist concentration, derived from the shift of the concentration–response curve in the presence of the antagonist. All numerical data in the concentration–response curves have been expressed as means $\pm$ standard error of the means (S.E.M). Each experiment was repeated on at least six preparations from three different animals. Statistical significance was determined by Student's *t*-test for unpaired samples (significance level $P < 0.05$).

2.2. Materials

(*R,S*)-Baclofen, CGP 52432 (3-[[3,4-dichloro-phenyl)-methyl]-amino]propyl](-P-diethoxymethyl)-phosphinic acid) and 3-APA (3-aminopropylphosphinic acid) were gifts from Ciba-Geigy (Basel, Switzerland). NO-711 (1-(2-((((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid) was purchased from Research Biochemicals (Natick, MA).

3. Results

In Mg^{2+}-free Krebs medium, rat neocortical slices exhibited repetitive spontaneous discharges. The frequency of these discharges was consistently reduced by 3-aminopropylphosphinic acid in a dose-dependent and reversible manner, over the concentration range of 50–1000 μM, there being no evidence of tissue desensitization to 3-aminopropylphosphinic acid upon prolonged exposure to this agonist. Also, the depression of spontaneous discharges in rat neocortical slice preparations induced by 3-aminopropylphosphinic acid was mediated through $GABA_B$ receptor sites, antagonised by the selective $GABA_B$ receptor antagonist CGP 52432 (3-[[3,4-dichlorophenyl)methyl]-amino]propyl](-P-diethoxymethyl)-phosphinic acid) originally developed by Froestl et al. (1992). In a typical experiment illustrated in Fig. 1a, 3-aminopropylphosphinic acid (3-APA; 70 μM) induced a suppression of discharge rate, reversibly antagonised by CGP 52432 (10 μM); recovery of the control response to 3-aminopropylphosphinic acid occurred within 30 min after tissue wash-out ($n = 8$). Neither the GABA uptake inhibitor NO-711 (5 and 10 μM) nor the $GABA_B$ receptor antagonist CGP 52432 (1, 5 and 10 μM) applied alone showed any effects on the control discharge rate. However, at 5 μM, NO-711 enhanced the depression of the spontaneous activity induced by 3-aminopropylphosphinic acid (3-APA; 70 μM), the enhanced response still being antagonised by 10 μM CGP 52432 ($n = 6$, Fig. 1b). The $GABA_B$ receptor agonist baclofen ($EC_{50} = 7$ μM) similarly reduced the frequency of the spontaneous activity, also antagonised by CGP 52432 (10 μM) ($n = 6$). However, as baclofen is not a substrate for GABA uptake carriers, its concentration–response curve was not affected by NO-711 (5 and 10 μM) ($n = 6$, data not shown). As shown in Fig. 1c, NO-711 (5 μM) did not alter the baclofen (BAC; 10 μM)-induced suppression of discharges.

From the 3-aminopropylphosphinic acid concentration–response curve shown in Fig. 2, the estimated EC_{50} value of 3-aminopropylphosphinic acid in suppressing the discharge rate was 66 μM, with a near

maximal effect at 700 μM ($n = 8$). By contrast, baclofen ($EC_{50} = 7$ μM) was about 10 times more potent than 3-aminopropylphosphinic acid in this depressant action, and reached a maximal effect at 80 μM ($n = 6$, data not shown). Pre-treatment of the slice with CGP 52432 (1, 5 and 10 μM) produced 2, 5 and 13 fold, rightward parallel shifts, respectively, of the 3-aminopropylphosphinic acid concentration–response curve without affecting the maximal response (mean $pA_2 = 6.0 \pm 0.1$; $n = 8$). This is illustrated in Fig. 2 by the 13 fold shift of the 3-aminopropylphosphinic acid concentration–response curve with CGP 52432 (10 μM) ($n = 8$). Again, the concentration–response curves to baclofen were similarly shifted to the right by CGP 52432 (1, 5 and 10 μM), giving a mean $pA_2 = 5.9 \pm 0.1$ ($n = 6$, data not shown).

In a further series of experiments, the concentration–response curves for 3-aminopropylphosphinic acid were potentiated by the GABA uptake inhibitor NO-711 (5 and 10 μM), with leftward shifts of the curves. This yielded a 2.9 fold shift with NO-711 (5 μM) (control $EC_{50} = 66$ μM, as against 23 μM with NO-711; $n = 6$, Fig. 2), and a 9 fold shift to 7.4 μM with NO-711 (10 μM) ($n = 6$). These potentiated responses were also sensitive to the $GABA_B$ receptor antagonist CGP 52432 (1, 5 and 10 μM) which caused parallel rightward shifts of the concentration–response curves for 3-aminopropylphosphinic acid in combination with NO-711 (5 and 10 μM) ($n = 6$ for each dose). Illustrative of this (Fig. 2), the curve for 3-aminopropylphosphinic acid together with 5 μM NO-711 was shifted 13 fold to the right by 10 μM CGP 52432 (3-aminopropylphosphinic acid with NO-711 $EC_{50} = 23$ μM, $n = 6$, as against 300 μM when applied together in the presence of CGP 52432, $n = 6$). This gave a mean $pA_2 = 6.0 \pm 0.1$, similar to the pA_2 value obtained in the absence of NO-711.

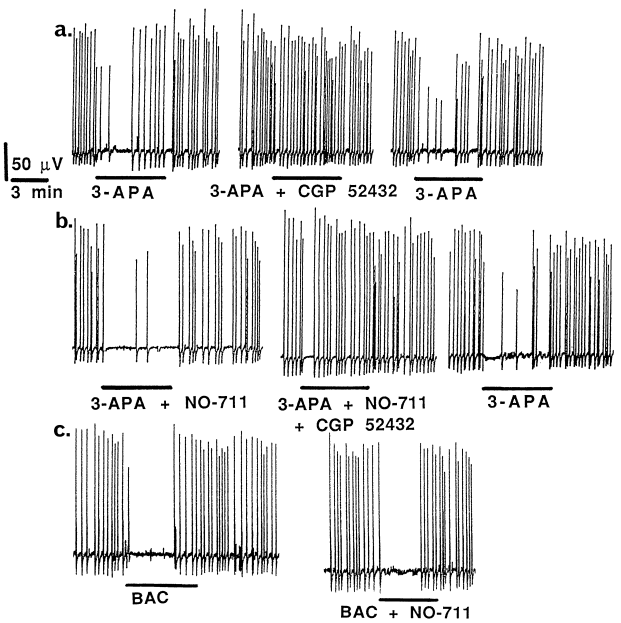

Fig. 1. A typical experiment showing suppression of spontaneous discharges by the $GABA_B$ receptor agonist 3-aminopropylphosphinic acid (3-APA) in the rat isolated neocortical slice preparation maintained in Mg^{2+}-free medium. (a) The 3-aminopropylphosphinic acid (3-APA; 70 μM)-induced depression of discharge rate was reversibly antagonised by the $GABA_B$ receptor antagonist CGP 52432 (10 μM). The control response to 3-aminopropylphosphinic acid was subsequently re-established upon washing out the antagonist ($n = 8$). (b) Pre-incubation of the same tissue with the GABA uptake inhibitor NO-711 (5 μM) enhanced the 3-aminopropylphosphinic acid (3-APA)-induced depressant action, antagonised by CGP 52432 (10 μM) with recovery after tissue wash-out ($n = 6$). (c) In a different preparation, NO-711 (5 μM) did not affect baclofen (BAC; 10 μM)-induced suppression of spontaneous discharges ($n = 6$).

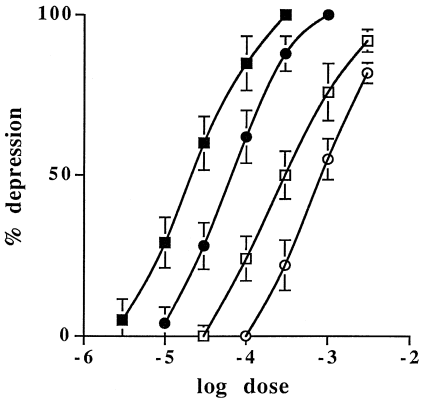

Fig. 2. Concentration–response curves for 3-aminopropylphosphinic acid-induced suppression of spontaneous discharges in the rat isolated neocortical slices, maintained in Mg^{2+}-free Krebs medium, in the absence (●) and presence (○) of CGP 52432 (10 μM), NO-711 (5 μM) (■) and CGP 52432 (10 μM) and NO-711 (5 μM) combined (□). Each individual value is expressed as a percentage depression of the average control discharge rate, and represents the mean and standard error of the mean of 6–8 determinations.

4. Discussion

In the present study, both 3-aminopropylphosphinic acid and baclofen depressed the frequency of spontaneous discharges in rat neocortical slice preparations maintained in Mg^{2+}-free Krebs medium in a concentration-dependent manner. These actions of 3-aminopropylphosphinic acid and baclofen were mediated through $GABA_B$ receptors since they were antagonised by the $GABA_B$ receptor antagonist CGP 52432 which yielded similar pA_2 values for each, indicating that the actions were mediated through the same receptor types. Although 3-aminopropylphosphinic acid was first said to be a more potent $GABA_B$ receptor agonist than baclofen in a variety of tissues (Dingwall et al., 1987; Hills et al., 1989; Pratt et al., 1989), previously we found it to be virtually inactive in the rat neocortex, up to a concentration of 10 μM (Ong et al., 1990b). It is now apparent that this concentration range originally used (Ong et al., 1990b) was too low, since the EC_{50} of 3-aminopropylphosphinic acid found here was 66 μM, as against 7 μM for baclofen, making it approxi-

mately 10 times less potent than baclofen. Such a low potency for 3-aminopropylphosphinic acid is unexpected, as it has been found to be either equipotent with, or more potent than baclofen in other central preparations (Seabrook et al., 1990; Ong et al., 1990a; Lovinger et al., 1992; Thompson and Gahwiler, 1992). However, the weak activity of 3-aminopropylphosphinic acid may be attributed to uptake of 3-aminopropylphosphinic acid by GABA transporters which limit the concentration accessible to the receptors in the slice. This explanation is strengthened by our finding that 3-aminopropylphosphinic acid-induced actions were potentiated by the GABA uptake inhibitor NO-711, a guvacine analogue which inhibits neuronal GABA uptake with an IC_{50} near 1 μM (Suzdak et al., 1992). The observed potentiation is unlikely to be due to an agonist action of NO-711 itself since the uptake blocker had no discernible effects on its own, or on responses to baclofen which is not a substrate for GABA transporters. Lovinger et al. (1992), using nipecotic acid, found no direct evidence that 3-aminopropylphosphinic acid is a substrate for uptake systems. We have no explanation for this discrepancy other than tissue differences (hippocampal vs. neocortical slices). The present results suggest that, particularly in slice preparations, the $GABA_B$ receptor agonist activity of 3-aminopropylphosphinic acid can be limited through uptake mechanisms, and may require the use of suitable blockers of the transporters in order to exhibit the full properties of 3-aminopropylphosphinic acid at $GABA_B$ receptor sites.

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References

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, J., Turnbull, M., 1980. (−) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283, 92–94.

Chapman, R.W., Danko, G., Prado, M.D., Egan, R.W., Kreutner, W., Rizzo, C.A., Hey, J.A., 1993. Further evidence for prejunctional GABA-B inhibition of cholinergic and peptidergic bronchoconstriction in guinea pigs: studies with new agonists and antagonists. Pharmacology 46, 315–323.

Dingwall, J.G., Ehrenfreund, J., Hall, R.G., Jack, J., 1987. Synthesis of γ-aminopropylphosphonous acids using hypophosphorous acid synthons. Phosphorus Sulfur 30, 571–574.

Froestl, W., Mickel, S.J., Von Sprecher, G., Bittiger, H., Olpe, H.R., 1992. Chemistry of new $GABA_B$ antagonists. Pharmacol. Commun. 2, 52–56.

Hills, J.M., Howson, W., 1991. The $GABA_B$ receptor profile of a series of phosphinic acids: Agonist and antagonist activity in a range of peripheral tissues. In: Erdo, S.L. (Ed.), GABA Outside the CNS. Springer-Verlag, Berlin, pp. 249–260.

Hills, J.M., Dingsdale, R.A., Parsons, M.E., Dolle, R.E., Howson, W., 1989. 3-Aminopropylphosphinic acid—a potent, selective $GABA_B$ agonist in the guinea-pig ileum and rat anococcygeus muscle. Br. J. Pharmacol. 97, 1292–1296.

Lacey, G., Curtis, D.R., 1994. Phosphinic acid derivatives as baclofen agonists and antagonists in the mammalian spinal cord: an in vivo study. Exp. Brain Res. 101, 59–72.

Lovinger, D.M., Harrison, N.L., Lambert, N.A., 1992. The actions of 3-aminopropanephosphinic acid at $GABA_B$ receptors in rat hippocampus. Eur. J. Pharmacol. 211, 337–341.

Ong, J., Harrison, N.L., Hall, R.G., Barker, J.L., Johnston, G.A.R., Kerr, D.I.B., 1990a. 3-Aminopropanephosphinic acid is a potent agonist at peripheral and central presynaptic $GABA_B$ receptors. Brain Res. 526, 138–142.

Ong, J., Kerr, D.I.B., Johnston, G.A.R., Hall, R.G., 1990b. Differing actions of baclofen and 3-aminopropylphosphinic acid in rat neocortical slices. Neurosci. Lett. 109, 169–173.

Pratt, G.D., Knott, C., Davey, R., Bowery, N.G., 1989. Characterisation of 3-aminopropylphosphinic acid as a $GABA_B$ agonist in rat brain tissue. Br. J. Pharmacol. 96, 141P.

Seabrook, G.R., Howson, W., Lacey, M.G., 1990. Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic $GABA_B$ receptors on neurones in rat brain slices. Br. J. Pharmacol. 101, 949–957.

Suzdak, P.D., Frederiksen, K., Erik-Andersen, K., Sorensen, P.O., Knutsen, L.J.S., Nielsen, E.B., 1992. NNC-711, a novel potent and selective γ-aminobutyric acid uptake inhibitor: pharmacological characterization. Eur. J. Pharmacol. 223, 189–198.

Thompson, S.M., Gahwiler, B.H., 1992. Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. J. Physiol. 451, 329–345.