δ-aminovaleric acid antagonizes the pharmacological actions of baclofen in the central nervous system

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Summary. The action of δ-aminovaleric acid (AVA) on the muscle relaxant properties of baclofen, a GABA$_B$ receptor agonist, was investigated in two experimental models: (1) the pathologically increased muscle tone of the gastrocnemius muscle in spastic mutant Han-Wistar rats and (2) the Hoffmann (H)-reflex recorded from plantar foot muscles after electrical stimulation of the tibial nerve in barbiturate (60 mg/kg) anaesthetized rats. In both paradigms co-administration of AVA (500 nmol/5 μl) antagonized the muscle relaxant action of intrathecally applied baclofen (0.2–2 nmol), but failed to affect the muscle relaxant effects of intrathecally injected muscimol (2–20 nmol). In contrast, coadministration of bicuculline (1 nmol) did block the muscle relaxant action of muscimol, but failed to alter the effects of baclofen. When administered alone, bicuculline (1 nmol), or AVA (500 nmol–2 μmol) were without intrinsic action in both paradigms. In an additional series of experiments we investigated the action of AVA on a supraspinal effect of baclofen. Co-administration of AVA (12.5 nmol/0.5 μl) in the ventromedial thalamic nucleus antagonized the catalepsy induced by baclofen (ED$_{50}$ 10 pmol/0.5 μl), as indicated by an increase in ED$_{50}$ of baclofen by a factor of 4.835 and a parallel shift of the probit-log dosage regression line to the right. The parallel shift seems to be consistent with a competitive mechanism of action of AVA. This study presents evidence that AVA antagonizes central pharmacological actions of baclofen at both spinal and supraspinal sites without affecting the actions of a GABA$_A$ agonist, muscimol.

Key words: δ-aminovaleric acid – Baclofen – GABA$_B$ antagonist – Spasticity

Introduction

Baclofen (β-(4-chlorophenyl)-γ-aminobutyric acid; Lioresal) is used clinically to reduce spasticity in various neurological disorders (Davidoff 1985; Young and Delwaide 1981). Its muscle relaxant action most probably results from a reduction in spinal reflex excitability, as baclofen has been shown to depress both monosynaptic and polysynaptic transmission in the spinal cord (Curtis et al. 1981; Davidoff and Sears 1974; Davies 1981; Fox et al. 1978; Pierau and Zimmermann 1973). In view of its close structural resemblance to the inhibitory neurotransmitter γ-aminobutyric acid (GABA), the drug was originally thought to be a GABA agonist. However, its depressant actions are generally not antagonized by bicuculline (Bowery et al. 1981; Curtis et al. 1974; Davies and Watkins 1974), a specific GABA antagonist (Johnston et al. 1972).

Recently, baclofen has been shown to be a selective ligand for a bicuculline-insensitive GABA receptor, termed the GABA$_B$ receptor (Hill and Bowery 1981), pharmacologically and anatomically distinct from the classical bicuculline-sensitive GABA$_A$ receptor (Hill and Bowery 1981; Bowery et al. 1980, 1981, 1983, 1984). The GABA$_B$ Receptor occurs in many parts of the central nervous system (CNS) including the thalamus and the spinal cord (Wilkin et al. 1981; Price et al. 1984; Bowery et al. 1984). Understanding of the physiological and pharmacological role of the GABA$_B$ receptor is hindered by the lack of specific antagonists (Bowery et al. 1983).
Δ-aminovaleric acid (AVA) has been proposed as a GABAB receptor antagonist in stimulated smooth muscle preparations such as the isolated rat anococcygeus muscle (Muhyaddin et al. 1982, 1983) and the guinea pig ileum (Kerr and Ong 1984). It shows, however, only weak activity as a GABAB receptor antagonist in the peripheral nervous system and seems also to act as a GABA_A receptor agonist (Bowery 1974). Recent results indicate that the GABA_B antagonistic action of AVA in the guinea pig ileum may be due to stimulation of GABA_A receptors (Allan and Dickenson 1986).

In the CNS, AVA has been shown to bind to both GABA_A and GABA_B receptors (Bowery 1983). A preliminary study on hippocampal slices suggested an antagonistic action of AVA at GABA_B sites in the CNS in vitro (Nakahiro et al. 1985). The present study was designed to investigate the possible GABA_B antagonistic action of AVA in the CNS in three in vivo paradigms. Emphasis was placed on the question of whether the possible GABA_B antagonistic action of AVA can be attributed to GABA_A mimetic properties of this drug (Allan and Dickenson 1986).

In order to study the effect of AVA on the central muscle relaxant action of baclofen in a state of pathologically increased muscle tone, the effect of intrathecal injections of AVA and baclofen was investigated in genetically spastic Wistar rats. This mutant strain of rats carries an autosomal recessive gene defect and exhibits within a critical age range an increased muscle tone which can be monitored by measuring spontaneous tonic activity in the electromyogram (EMG) of the gastrocnemius muscle (Pitterman et al. 1976; Schwarz et al. 1985).

As baclofen is thought to exert its antispastic action by suppressing spinal reflex transmission (Curtis et al. 1981; Davidoff and Sears 1974; Davies 1981; Fox et al. 1978; Pierau and Zimmermann 1973) we have further investigated whether or not intrathecal administration of AVA antagonized the baclofen induced depression of the Hoffman (H)-reflex in anaesthetized rats. In man (Magladery and McDougal 1950), transcutaneous electrical stimulation of the tibial nerve elicits a short-latency EMG wave in the triceps surae muscle due to direct excitation of axons of α-motoneurones, designated as the muscle-(M-)wave. The M-wave is followed by an EMG wave of longer latency, designated as the H-reflex. This latter response has been attributed to reflex excitation of spinal α-motoneurones predominantly by muscle spindle afferent fibres. In rats, a reflex response of comparable properties can be recorded in plantar foot muscles after electrical stimulation of the tibial nerve (Meinck 1976).

Although the muscle relaxant action of baclofen is thought to lie in the spinal cord, supraspinal effects of baclofen have also been reported in vivo (Curtis et al. 1974; Davies and Watkins 1974; DiChiara et al. 1979; Lanthorn and Cotman 1981; Levy and Proudfoot 1979; Newberry and Nicoll 1984; Olpe et al. 1977; Wüllner et al. 1987). Local injection of picomolar amounts of baclofen into the ventromedial thalamic nucleus (VM) of the rat induces catalepsy (DiChiara et al. 1979; Wüllner et al. 1987), a behavioural state in which animals fail to correct externally imposed postures. This behavioural phenomenon was used to investigate the possible antagonistic effect of AVA on the action of baclofen at supraspinal sites.

### Material and methods

Throughout this study each animal was used only once.

#### Tonic EMG activity of gastrocnemius muscle in spastic Han-Wistar rats

Male and female genetically spastic rats (Han-Wistar: spa-spa; Zentralinstitut für Versuchstierkunde, Hannover, FRG), 120–180 g in weight, were chronically equipped with intrathecal polyethylene catheters (PE 10) (Yaksh and Rudy 1976) under pentobarbitone anaesthesia (40 mg/kg i.p.). Briefly, the atlanto-occipital membrane was exposed and a small incision made. The catheter was then inserted and advanced to the lumbar enlargement of the spinal cord. The catheter was attached to the skull by means of stainless steel anchor screws and rapid-setting dental acrylic. After a recovery period of 1–2 days the intrathecal injections were performed in unanesthetized rats. The drugs were delivered in a volume of 5 μl at a rate of 1 μl/min. Injections were followed by 10 μl of vehicle to clear the catheter of the drug. After performance of experiments, the correct location of the catheter tip was verified by an injection of 2% Evans blue dye through the catheter.

Spontaneous tonic EMG activity was recorded from the gastrocnemius muscle of genetically spastic rats with pairs of teflon-insulated stainless-steel wire electrodes (Cooner Wire, Chabworth, USA, AS 632 SS) inserted percutaneously into the muscle. The rats were placed separately in ventilated Plexiglas boxes and their hindlimbs, which were gently secured with adhesive tape, were allowed to hang through slots in the bottom of the boxes. The electrical signals were amplified, band-pass filtered (5 Hz–10 kHz), fullwave rectified, and integrated. The EMG was recorded continuously and the integrated activity was determined over 10 min periods. The spontaneous predrug control activity in the EMG of the gastrocnemius muscle was measured in two 10 min periods. The changes in the activity in the EMG after intrathecal injection of solvent or drugs were expressed as a percentage of the mean value calculated from the two preinjection (control) measurements. Statistical evaluation was carried out using the Student's t-test.

#### H-reflex

Male Wistar rats (F. Winkelmann, Borchen, FRG), 280–330 g in weight, were used. Animals were equipped, under pentobarbitone