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IN VIVO EFFECTS OF AMINOOXYACETIC ACID AND VALPROIC ACID ON NERVE TERMINAL (SYNAPTOSOMAL) GABA LEVELS IN DISCRETE BRAIN AREAS OF THE RAT

CORRELATION TO PHARMACOLOGICAL ACTIVITIES*

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Abstract—A newly developed synaptosomal model was used to evaluate the *in vivo* effects of the GABA-elevating drugs aminooxyacetic acid (AOAA, 30 mg/kg i.p.) and valproic acid (VPA, 200 mg/kg i.p.) on GABA levels in nerve endings of 11 brain regions in rats as a function of time after administration. The data obtained were compared with the magnitude and time course of the effects of both drugs in rats on body temperature, pain response and against seizures induced by electroshock, pentylenetetrazol and 3-mercaptopropionic acid. Following AOAA, maximum increases in synaptosomal GABA levels of brain regions were observed 6 hr after administration. At this time, GABA was significantly elevated up to 300% over control values in synaptosomal fractions from all 11 regions. However, the hypothermic and antinociceptive effects of the drug as well as its anticonvulsant action against electroshock and pentylenetetrazol induced seizures were maximal 1 hr after injection and had vanished after 6 hr, i.e. at the time of maximum GABA increases in synaptosomes. The only pharmacological effect of AOAA which paralleled the time course of the synaptosomal GABA elevation was the attenuation of seizures induced by 3-mercaptopropionic acid. Following VPA, the effect on synaptosomal GABA levels was much more rapid in onset and significant increases were already determined 5 to 30 min after administration. Significant increases of up to 80% over control values were found in synaptosomal fractions from olfactory bulb, frontal cortex, hippocampus, hypothalamus, tectum, substantia nigra and cerebellum. In contrast to AOAA, the time course of the synaptosomal GABA increases, at least in some regions, was similar to the time course of VPA's antinociceptice effects and its anticonvulsant effects in the three seizure models studied. The data may suggest that AOAA and VPA increase different pools of GABA within nerve terminals, only one of which is involved in GABA-mediated neurotransmission.

Several characteristics of the γ-aminobutyric acid (GABA) system complicate our understanding of the role of this neurotransmitter in brain functions. These characteristics include the regional distribution of GABA in the brain, the types of circuits in which GABA containing neurons are found, and the high degree of cellular compartmentation of GABA in brain tissue [cf. ref. 1]. Synthetic and degradative enzymes for GABA, as well as high affinity uptake mechanisms, are present in many cells, including GABAergic neurons, non-GABAergic neurons, and glia cells. As a consequence, the degree to which drug-induced changes in brain GABA can be expected to influence GABA-mediated transmission is difficult to ascertain. In biochemical and pharmacological studies on drugs which alter GABA levels in the brain, it thus seems important to determine the effect of the treatment on GABA and its metabolism at the subcellular level with particular attention being placed on events occurring in nerve endings. Since the direct measurement of *in vivo* changes of GABA in GABAergic nerve terminals is not possible to date, several laboratories, including our own, have used synaptosomal fractions from the brains of drug-treated rodents for estimation of changes in nerve terminal GABA induced by the treatment *in vivo* [2–12]. More recently, these "synaptosomal models" for determination of drug-induced *in vivo* change of nerve terminal GABA in the brain have been improved in order to allow the measurement of synaptosomal GABA levels in various discrete brain regions of rats [13, 14]. By means of such a newly developed technique [13], we have studied the effects of 2 widely used GABA-elevating drugs, namely aminooxyacetic acid (AOAA) and the antiepileptic valproic acid (VPA), on synaptosomal GABA levels in 11 rat brain regions. The magnitude and time course of the synaptosomal GABA changes was compared with a number of pharmacological effects of both drugs in rats. This study was prompted by previous experiments [15], in which the marked differences in pharmacological effects between VPA and AOAA could not be related to their differential

* Dedicated to Prof. Helmut Kewitz on occasion of his 65th anniversary.

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effects on GABA levels in whole tissue of discrete brain regions. Some of the present results have been reported in preliminary form elsewhere [16].

MATERIALS AND METHODS

Drugs. Aminooxyacetic acid hemihydrochloride (AOAA) was purchased from Sigma (Munich, F.R.G.). Valproic acid (VPA), used as the sodium salt, was kindly provided by Desitin-Werk Carl Klinke (Hamburg, F.R.G.). Pentylenetetrazol was obtained from Knoll AG (Ludwigshafen, F.R.G.) and 3-mercaptopropionic acid (3-MP) from Merck (Darmstadt, F.R.G.). Drugs were freshly dissolved in distilled water and injected in a volume of 2 ml/kg. All doses refer to the forms of drugs listed above. Control animals received 2 ml/kg saline.

Animals. All experiments were carried out in female Wistar rats (Winkelmann Versuchstierzuchtanstalt, Borchen, F.R.G.) weighing 200–220 g. Rats were kept in groups of 10 in Makrolon® cages at constant temperature (25°) and controlled humidity (*ca.* 50%) with a 12 hr light cycle beginning at 0700 hr, and were fed Altromin® 1324 standard food (Altromin, Lage, F.R.G.). All experiments were carried out in the forenoon at 25°.

Determination of anticonvulsant activity. Anticonvulsant activity of AOAA (30 mg/kg i.p.) and VPA (200 mg/kg i.p.) was determined at different times after administration using three seizure models, namely the threshold for maximal (tonic extension) electroconvulsions, seizures induced by pentylenetetrazol, and seizures induced by 3-mercaptopropionic acid (3-MP).

The threshold for maximal electroconvulsions was determined in groups of 15 rats. Electroshock was applied by eye electrodes using a Layfayette A-615 B shocker (Lafayette Instrument Co., Indiana, U.S.A.). Stimulation data were 50 Hz for 0.2 sec with the serial resistance of the apparatus set to 10 kΩ. The extension of the hind limbs was taken as the endpoint. The threshold was determined by the "up and down" method of Kimball *et al.* [17] and calculated as the voltage inducing the extensor phase in 50% of the rats (EV 50). All experimental groups were compared with concurrent control groups. Each control or experimental group was used for only one threshold determination.

In groups of 10 rats, pentylenetetrazol (70 mg/kg) was injected subcutaneously. Following the injection, animals were observed for 30 min for the occurrence of seizures which were rated as follows: 0, no seizure; 0.5, clonic seizure, animal remains on its feet; 1, clonic-tonic seizure with loss of righting reflexes. Intermittent twitching was generally disregarded. The maximum scores of the 10 animals were added up and used for comparison between control groups and experimental groups.

In further groups of 10 rats, 3-MP (50 mg/kg i.p.) was administered and the animals were observed during the following 30 min for the occurrence of seizures. The seizure severity was rated as follows: 0, no seizure; 1, running seizures; 2, clonic seizures, animal remains on its feet; 3, clonic seizures with loss of righting reflexes; 4, tonic forelimb extension; 5, tonic hindlimb extension. The maximum score for each animal was recorded and the mean of the 10 animals was used for comparison with controls.

Determination of pain response and body temperature. The antinociceptive effect of AOAA (30 mg/kg i.p.) and VPA (200 mg/kg i.p.) was determined in groups of 15 rats at different times after administration using a "hot plate" with 56°. The time in seconds to the first licking of the paws was recorded. Each group of animals was used only once. Immediately before the hot plate test, rectal temperature was recorded by means of an electrical thermometer (Ellab Instruments, Copenhagen, Denmark). All experimental groups were compared with concurrent control groups.

GABA determination in whole tissue and synaptosomal fractions of 11 rat brain regions. For the biochemical determinations, groups of 10 rats were killed at different times after administration of VPA (200 mg/kg i.p.) or AOAA (30 mg/kg i.p.). Five rats of each group were used for GABA determinations in whole tissue of brain regions and five animals were used for GABA analysis in synaptosomal fractions of the respective regions. GABA concentrations in each treated group were compared with those in a control group of 10 rats which were killed immediately prior to the treated animals. Rats were killed by decapitation and the brains were rapidly removed and dissected on a cold plate at −18° (Leitz Kryomoat, Wetzlar, F.R.G.) within 4 min after decapitation into the following 11 regions: olfactory bulb, frontal cortex, corpus striatum, hippocampus, thalamus, hypothalamus, tectum (superior and inferior colliculus), substantia nigra, pons, medulla oblongata, and cerebellum. Details of the dissection technique have been published elsewhere [13], and it has been demonstrated that no postmortem GABA increases occur during rapid dissection at −18° [13]. For whole tissue GABA determinations, the individual regions were rapidly homogenized in 2 ml of ice-cold 80% ethanol in order to destroy any enzyme which could alter GABA levels *in vitro*. For preparation of synaptosomal fractions, the regions were rapidly homogenized in 0.32 M sucrose (pH 7.0) containing 1 mM 3-MP, an inhibitor of GABA synthesis [18], in order to prevent *in vitro* increases of GABA during homogenization and fractionation procedures [cf. 13]. In order to save preparation time, 1 ml of sucrose was used for homogenisation of the individual regions irrespective of the tissue weight. Although homogenates therefore deviated in part from the 10% w/v normally used for synaptosome preparation (actual tissue concentrations varied from 2–12.5%), direct comparison with 10% w/v homogenates of the 11 brain regions showed no significant difference in the protein yield of the synaptosomal fractions obtained by both procedures. After homogenization, further processing of samples was done as shown in Fig. 1. Homogenization and all subsequent steps were carried out at 0–4°. Details of the subcellular fractionation, the characterization of the synaptosomal fractions by electron microscopy, and the various control experiments carried out during development of the synaptosome preparation have been described elsewhere [13]. Briefly, it was shown that (1) in synaptosomal fractions prepared from the 11 brain regions from rat brain by the procedure

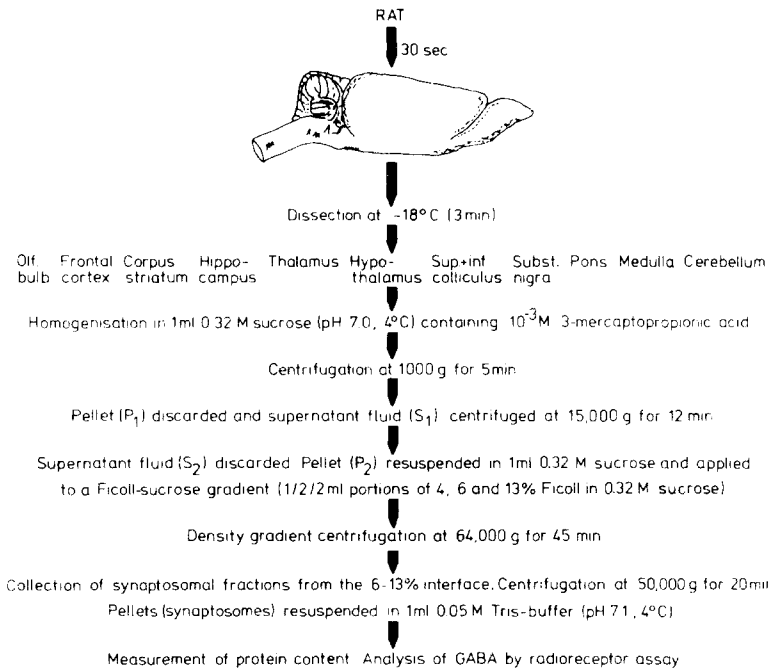

Fig. 1. Scheme for isolation of synaptosomal fractions from 11 regions of one rat brain.

shown in Fig. 1, synaptosomes accounted for the majority of structures which could be identified; (2) postmortem increases of GABA during removal and dissection of brain tissue, homogenization and fractionation procedures could be sufficiently minimized by rapid processing of the tissue at low temperatures and inclusion of 3-MP in the homogenizing medium; (3) GABA degradation was not active during the fractionation procedures at 0–4°; and (4) high concentrations of GABA added to the homogenizing medium did not significantly increase the GABA content in synaptosomes, thereby indicating that no redistribution of GABA was occurring during the fractionation procedures.

GABA levels in whole tissue homogenates of brain regions were measured by the enzymatic "GABAase" method as described recently [13]. The very low levels of GABA in synaptosomal fractions were determined by a sensitive and specific radioreceptor assay [19]. Protein content of whole tissue homogenates and synaptosomal fractions was determined by the method of Lowry *et al.* [20] as modified by Markwell *et al.* [21].

Statistics. Arithmetical means and S.E. are given for the pharmacological and biochemical determinations. The electroconvulsive threshold is given as EV 50 with confidence limits for 95% probability. Significance of differences was calculated by comparing each treated group with a concurrent control group by Student's *t*-test.

RESULTS

Control GABA levels in whole tissue and synaptosomal fractions of rat brain regions

Control GABA levels determined in 30 rats are shown in Table 1. In whole tissue of the 11 brain regions, the highest values were found in hypothalamus, substantia nigra, and olfactory bulbs, whereas synaptosomal GABA levels were highest in tectum, olfactory bulb and corpus striatum. In general, the synaptosomal GABA levels were considerably lower than whole tissue levels when expressed on a nmole per mg protein basis, which may be explained, at least in part, by a loss of GABA from synaptosomes during the subcellular fractionation procedures. Determinations of GABA in synaptosomal fractions thus obviously cannot provide an absolute measure of GABA concentrations present in nerve endings of the respective regions in the intact animal but, as shown in previous studies [13–15], they can be used for estimation of drug-induced *in vivo* changes of nerve terminal GABA in discrete brain areas.

Effect of AOAA and VPA on GABA levels in whole tissue and synaptosomal fractions of rat brain regions

The effect of AOAA (30 mg/kg i.p.) and VPA (200 mg/kg i.p.) on GABA levels in whole tissue and synaptosomal fractions of 11 brain regions in rats is shown in Figs 2 and 3. Both drugs caused significant GABA increases when compared with concurrent controls, but the time course, magnitude, compartmentation, and profile of the increases across areas generated by AOAA were quite distinct from those obtained with VPA.

One hour following AOAA injection, the most marked GABA elevation in both whole tissue and synaptosomal fractions was seen in frontal cortex and olfactory bulbs (Fig. 2). Significant whole tissue GABA increases were also determined in hippocampus, hypothalamus, substantia nigra, and cerebellum; however, in these regions AOAA exerted no significant effect on synaptosomal GABA levels.

Table 1. Control GABA levels in whole tissue and synaptosomal fractions of 11 rat brain regions

Region	GABA (nmole/mg protein)	
	Whole tissue	Synaptosomal fraction
Olfactory bulb	24.7 ± 0.7	10.4 ± 0.6
Frontal cortex	9.4 ± 0.5	7.8 ± 0.3
Corpus striatum	16.8 ± 0.5	10.1 ± 0.5
Hippocampus	11.6 ± 0.4	6.6 ± 0.3
Thalamus	24.0 ± 0.7	9.0 ± 0.5
Hypothalamus	31.6 ± 0.5	8.2 ± 0.5
Tectum	18.6 ± 0.4	17.6 ± 0.8
Substantia nigra	26.8 ± 0.8	5.1 ± 0.3
Pons	8.1 ± 0.2	3.8 ± 0.2
Cerebellum	7.6 ± 0.2	3.7 ± 0.2
Medulla oblongata	7.3 ± 0.2	6.3 ± 0.3

For whole tissue determinations, the regions were homogenized in 80% ethanol immediately after dissection. For preparation of synaptosomal fractions, tissue samples were homogenized in 0.32 M sucrose containing 1 mM 3-mercaptopropionic acid and further processed as outlined in Fig. 1. Data are means ± S.E. of 30 saline-treated control rats.

Two hours after AOAA, the whole tissue GABA increases had become more pronounced in most regions and, in addition to olfactory bulb and cortex, significant synaptosomal GABA increments were now observed also in hippocampus, thalamus, and cerebellum. However, the synaptosomal GABA content of the midbrain regions remained to be unchanged. Between 2 and 6 hr after administration of AOAA, GABA levels in synaptosomes rose dramatically, and significant increases up to 300% over control values occurred in all regions including the midbrain areas. In contrast to the synaptosome GABA content, whole tissue GABA increases at 6 hr did not change significantly from the increases at 2 hr post-injection except in frontal cortex and hippocampus.

Following administration of VPA, the effect on synaptosomal GABA levels was much more rapid in onset but shorter-lasting compared to AOAA (Fig. 3). Thus, significant GABA elevations in synaptosomes were already determined 5 min after VPA injection in cortex (44% over controls), hippocampus (64%), hypothalamus (80%), and tectum (60%), whereas significant whole tissue GABA increases at this time were only observed in the hypothalamus. Thirty minutes post-injection, GABA levels were significantly elevated in synaptosomal fractions from olfactory bulb, hypothalamus, tectum, substantia nigra, and cerebellum, again the most marked effect (80% over control) being determined in hypothalamus. As after 5 min, in most regions the relative increases in synaptosomal GABA were considerably larger than those determined in whole tissue. Two hours after injection of VPA, GABA levels had returned to control values in the endbrain areas. In the other regions, there were still moderate GABA increases in synaptosomal fractions which, however, were only significant in the tectum.

Both AOAA and VPA did not significantly alter the amount of material found in the synaptosomal fractions as gauged by their protein content.

Pharmacological effects of AOAA and VPA in rats

The administration of AOAA (30 mg/kg i.p.) to rats produced a profound decrease in body temperature which was maximal after 1 hr (Fig. 4). Two hr post-injection, the hypothermia was less marked but still significant, whereas after 6 hr rectal temperature had approached control values. In the hot plate test, AOAA exerted a very pronounced antinociceptive effect which became maximal 2 hr after administration but had disappeared after 6 hr (Fig. 4). The anticonvulsant efficacy of AOAA in rats was determined by three different seizure models, namely the maximal electroshock seizure (MES) threshold, and seizures induced by pentylenetetrazol and 3-MP. As shown in Fig. 4, a significant increase in the MES threshold was observed 1 hr following AOAA treatment, but thereafter the threshold declined even below control values. A similar time course was determined in terms of the effect on pentylenetetrazol-induced seizures, which were attenuated only at 1 hr after AOAA injection. In contrast, seizures induced by 3-MP were not affected by AOAA after 1 and 2 hr but significantly attenuated at 6 hr.

Following i.p. injection of VPA (200 mg/kg), rectal temperature was only moderately changed compared to AOAA (Fig. 5). Thus, a small but significant increase in temperature was found after 30 min, whereas 2 hr following VPA administration, a moderate decline in body temperature was observed. In the hot plate test, VPA displayed an antinociceptive effect which was more rapid in onset and shorter-lasting compared to AOAA (Fig. 5). The maximum effect, determined 5 min after VPA injection, was almost equal to that seen 1–2 hr after AOAA. As regards anticonvulsant efficacy, VPA was clearly superior to AOAA in all three seizure models

employed (Fig. 5). Peak anticonvulsant activity occurred 5–30 min after VPA administration, and 2 hr post-injection there was still a significant anticonvulsant effect against electroshock and 3-MP induced seizures.

DISCUSSION

In the present study, a recently developed synaptosomal model for determination of drug-induced *in vivo* changes in nerve terminal GABA levels of discrete brain regions [13] was used to evaluate the

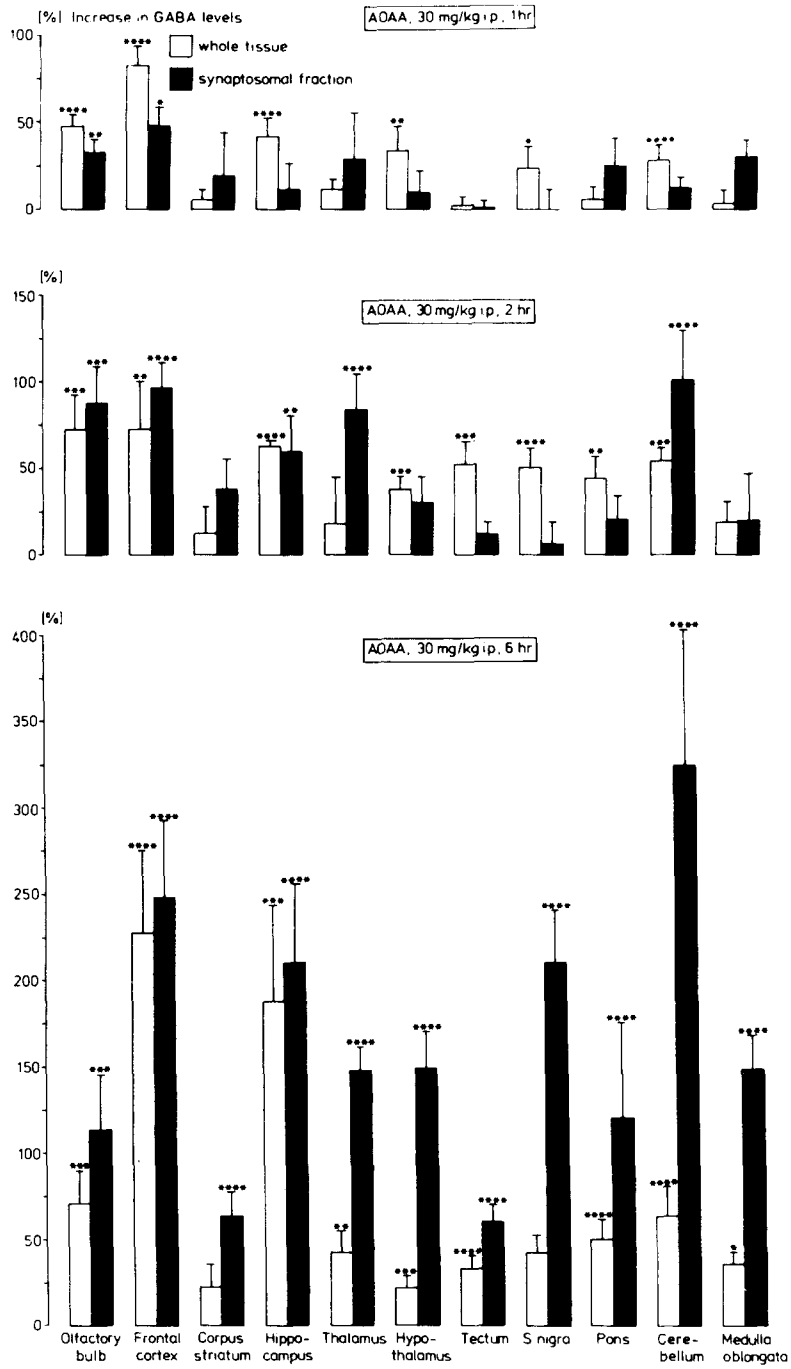

Fig. 2. Effect of AOAA on GABA levels in whole tissue and synaptosomal fractions of 11 rat brain regions following 1, 2, and 6 hr after i.p. administration of 30 mg/kg. Results are expressed as percentage increase (means ± S.E. of five rats) over concurrent control determinations (see Table 1 for absolute control values). Significance of differences to the concurrent control group is indicated by $^{*}P < 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$, and $^{****}P < 0.001$.

effects of 2 GABA-elevating drugs, i.e. AOAA and VPA, on synaptosomal GABA content as a function of time after administration. Besides the biochemical measurements, the effects of both drugs on seizure excitability, pain response and body temperature was compared.

Although VPA and AOAA are known to exert anticonvulsant effects in various animal models of seizure states [cf. ref. 22], it remains a matter of dispute as to whether the anticonvulsant activity of these drugs is related to their GABA-elevating effect. Thus, the degree of brain GABA increases that occurs with anticonvulsant doses of VPA and AOAA strikingly differs in that anticonvulsant activity of VPA in rodents is seen with whole brain GABA increments in the range of 30–60% over

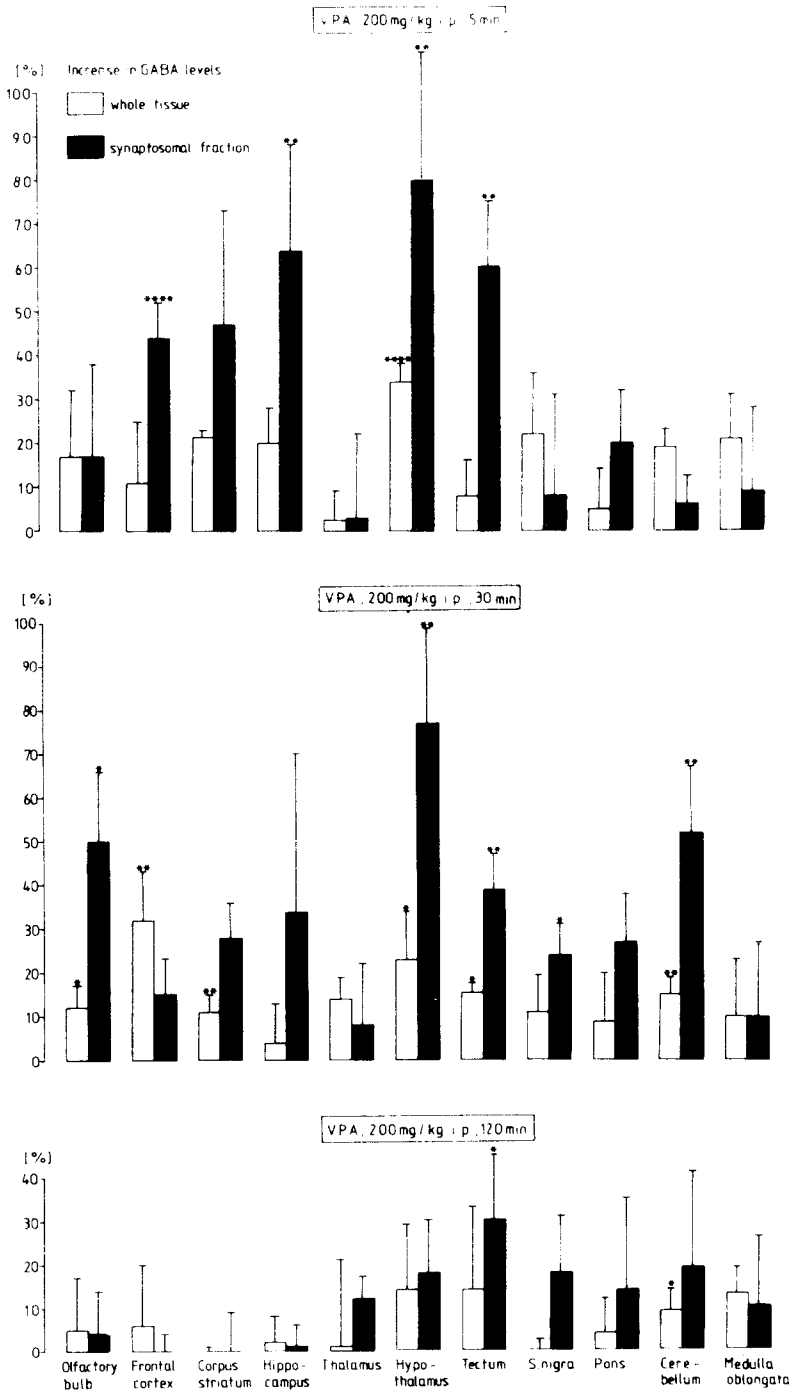

Fig. 3. Effect ov VPA on GABA levels in whole tissue and synaptosomal fractions of 11 rat brain regions following 5, 30, and 120 min after i.p. administration of 200 mg/kg. For further explanation see the legend to Fig. 2.

controls, whereas antiseizure actions of AOAA only occur with much higher GABA increases [e.g. ref. 23]. Furthermore, in mice with audiogenic seizures VPA has been shown to exhibit anticonvulsant properties already at doses (200 mg/kg i.p.) that do not significantly increase whole brain GABA levels [24]. In rats, VPA has been reported to exert its maximum anticonvulsant effect against electroshock convulsions within 5–15 min after i.p. administration, whereas the first significant elevation of cerebral GABA was seen only after 30 min [25]. However, all these studies have failed to take into account possible regional variations in VPA's and AOAA's effects on GABA levels, which are concealed by whole brain measurements. Actually, experiments by Iadarola *et al.* [26] and our group [15] in rats have indicated that VPA is equally or even more effective than AOAA in raising GABA levels in certain discrete brain regions, e.g. in superior colliculus and hypothalamus, when both

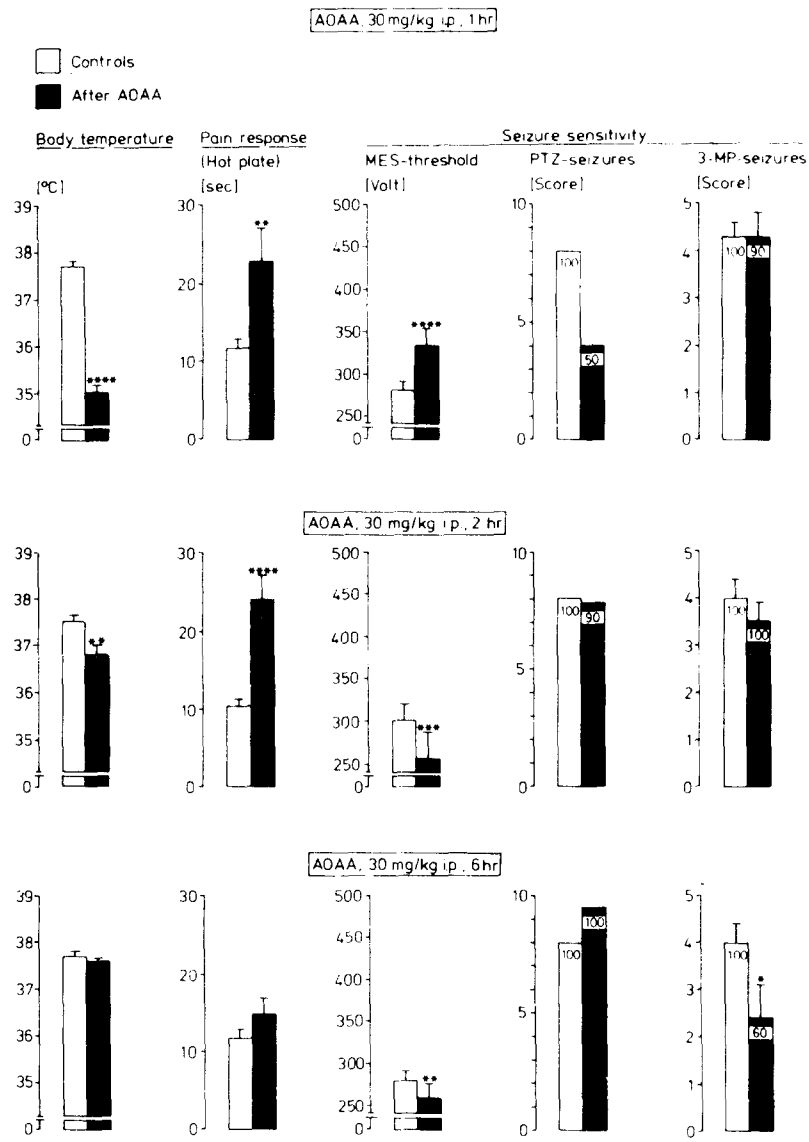

Fig. 4. Effect of AOAA on rectal temperature, pain response and seizure susceptibility in rats following 1, 2, and 6 hr after i.p. administration of 30 mg/kg. Data for body temperature and pain response are means ± S.E. of 15 rats per group. The pain response was determined in the hot plate test as the time in sec to the first licking of the paws. The threshold for maximal electroshock seizures (MES) was determined in groups of 15 rats as the voltage inducing an extension of the hind limbs in 50% of the rats (EV 50 with confidence limits for 95% probability). The scores for pentylenetetrazol (PTZ) and 3-mercaptopropionic (3-MP) induced seizures were derived from the scales that we used to rate the severity of the seizures (see Materials and Methods). Ten rats were used per group. The figures on the PTZ and 3-MP bars indicate the percentage of animals in which seizures occurred. Significance of differences to concurrent control groups is indicated by $^{*}P < 0.05$, $^{**}P < 0.02$, $^{***}P < 0.01$, $^{****}P < 0.001$.

drugs are administered at doses with comparable anticonvulsant activity. Furthermore, AOAA and VPA have been reported to differ also in their effect on the compartmentation of GABA in that VPA preferentially increases the GABA concentration in nerve terminals while AOAA seem to exert its predominant effect on GABA levels in non-nerve terminal compartments [6, 13, 27]. In fact, it was shown in mice that significant synaptosomal GABA increases already occur following 100–200 mg/kg VPA [9], i.e. doses which had been previously reported not to elevate brain GABA in mice [24]. Thus, it is evident that whole brain measurement of GABA is not an appropriate parameter to use in an attempt to correlate elevation of GABA levels and neuropharmacological effects such as anticonvulsant action.

In the present study, we could resolve some of the above mentioned discrepancies surrounding VPA's effects on GABA levels and seizure excitability. As already reported for the substantia nigra by Iadarola and Gale [27], VPA significantly increased GABA levels in nerve terminals (synaptosomes) of several discrete brain regions, whereas the relative increases in whole tissue GABA of these regions were less marked. Actually, determination of GABA only in whole tissue of the respective regions would have concealed the profound changes induced by VPA in the nerve terminal GABA compartment. Significant increases in synaptosomal GABA content were induced by VPA already 5 min after the injection, thus refuting the assumption of Kerwin *et al.* [25] and others that VPA does not increase GABA levels within such short time intervals. When the time

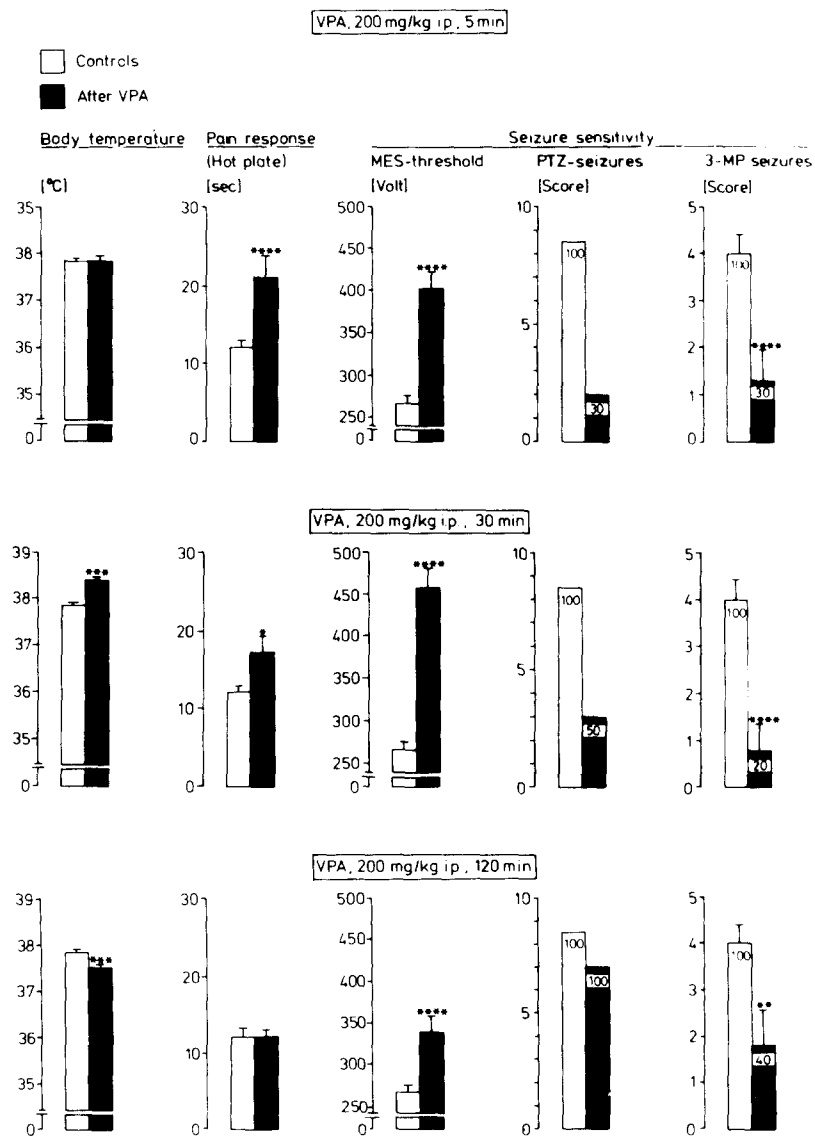

Fig. 5. Effect of VPA on rectal temperature, pain response and seizure susceptibility in rats following 5, 30, and 120 min after i.p. administration of 200 mg/kg. For further explanation see the legend to Fig. 4.

course of the synaptosomal GABA changes after VPA was compared with the temporal pattern of the anticonvulsant effects of the drug, the GABA increases in hypothalamus and tectum appeared to correlate best with VPA's action in the three seizure models studies. Similarly, the temporal pattern of the antinociceptive effect of VPA was comparable to that of the synaptosomal GABA elevation in the hypothalamus, a region which is thought to play an important role in mediating antinoceptive drug action [28]. However, such an attempt to pinpoint particular brain regions which may be important for VPA's pharmacological effects is biased by variations in the population density of nerve endings containing GABA. In other words, only a portion of the synaptosomes isolated under the conditions of the present study arises from GABAergic nerve terminals and this portion varies from region to region. This necessarily leads to underestimates of VPA's effects on nerve ending GABA, and the extent of these underestimates differs among brain regions. In any event, the present data clearly demonstrate that VPA rapidly increases synaptosomal GABA levels in several brain regions and that the time course of this effect in some of these regions is very similar to the time course of the pharmacological effects of the drug.

A strong objection to the GABA hypothesis for VPA has been that an increase in presynaptic GABA levels not necessarily leads to an increase in GABA release and thus potentiation of GABA mediated synaptic transmission [29]. However, more recent studies with cerebrospinal fluid GABA measurements in dogs and epileptic patients have strongly indicated that marked increases in GABA release occur in response to VPA administration [30, 31].

The mechanism by which VPA increases nerve terminal GABA is unsettled. VPA has been shown to dose-dependently decrease the GABA degrading enzyme GABA-oxoglutarate aminotransferase (GABA-T; E.C. 2.6.1.19) in synaptosomal fractions from mouse brain *in vivo* [9], but this effect was only moderate (10–25% inhibition) if one takes into consideration that *in vivo* there is a spare capacity of the enzyme of approx. 50% [32]. On the other hand, administration of 100–200 mg/kg VPA in mice has been reported to increase GABA synthesis by 40–90% [33], most likely by activation of glutamic decarboxylase (GAD; E.C. 4.1.1.15) [9, 34], which could explain the rapid onset of the GABA increases found in the present study. Higher doses of VPA ($\geq$ 400 mg/kg i.p.) were found to decrease GABA turnover in brain cortex of mice and rats [35, 36] which might be interpreted as a feedback mechanism to the potentiation of postsynaptic GABA responses reported after high (> 1 mM) concentrations of VPA [37]. However, it should be considered that the doses of VPA necessary to decrease GABA turnover in these experiments were within the neurotoxic dose range (median neurotoxic doses of VPA in rats and mice are about 300–500 mg/kg i.p.; [38] so that the biochemical changes may not be related to the anticonvulsant effect of this drug.

As regards AOAA, a potent inhibitor of GABA-T, the effects of this compound on synaptosomal GABA levels in brain regions of rats differed in several aspects from those observed with VPA in the present study. Thus, between 1 and 2 hr following administration, AOAA induced synaptosomal GABA increases in the endbrain areas, the thalamus, and the cerebellum, whereas GABA levels were not altered in synaptosomes from hypothalamus and the midbrain and hindbrain regions. These results are consistent with data by Iadarola and Gale [27] who found in rats that 2 hr after 30 mg/kg AOAA the nerve terminal GABA content of the substantia nigra was not altered. However, the situation completely changed when synaptosomal GABA levels were determined 6 hr following AOAA injection. At this time, marked elevations of synaptosomal GABA were found in all regions including the substantia nigra, although in most areas the whole tissue GABA levels had not further increased between 2 and 6 hr. Similar observations have been recently reported for gabaculine, another potent GABA-T inhibitor, which caused 3 to 4 fold increases in synaptosomal GABA content of different rat brain regions, but in diencephalon and mesencephalon these increases occurred much later than in other areas [14]. This may suggest that either the sensitivity of the nerve terminal GABA-T to GABA-T inhibitors differs among brain regions or the time by which the drugs reach and enter nerve terminals differs from region to region.

When one compares the magnitude and time course of the synaptosomal GABA changes caused by AOAA with those of the pharmacological effects of the drug in rats, several discrepancies are conspicuous. Anticonvulsant effects on electroshock and pentylenetetrazol seizures were present 1 hr after AOAA injection but vanished thereafter although synaptosomal GABA levels continued to rise in all brain regions. The same discrepancy applied to the marked effects of the drug on body temperature and pain response which had subsided 6 hr after administration despite maximum GABA increases in synaptosomes. The only pharmacological effect of AOAA which parelleled the rise in synaptosomal GABA levels was the attenuation of seizures induced by 3-MP, an inhibitor of GAD which is thought to cause seizures by the induced decrease of GABA in nerve terminals [39, 40]. In fact, the maximum anticonvulsant effect of AOAA against 3-MP was found 6 hr after administration; however, even then AOAA was less potent than VPA to attenuate these seizures although it was much more potent than VPA to increase synaptosomal GABA at this time.

There are at least two possible explanations for these inconsistencies:

(1) Recent studies by Abe and Matsuda [41] strongly suggest the existence of two different GABA pools in nerve terminals. One pool consists of newly synthesized GABA and waits in cytoplasm (probably near the presynaptic membrane) to be released into the synaptic cleft. The pool is associated with GAD and is not under the influence of GABA-T. The second pool consists of newly taken up GABA from the synaptic cleft and stays in synaptic vesicles (possibly near synaptic mitochondria) to be degraded. This pool is closely under the influence of GABA-T. Treatment of mice with AOAA (30 mg/kg i.p.) was shown to enhance the level of newly taken up GABA about 3-fold, whereas it had no

effect on the level of newly synthesized GABA [41]. These considerations could explain the present finding in rats that large increases of synaptosomal GABA by AOAA are not associated with profound functional effects. On the other hand, increase of GABA synthesis by VPA through activation of GAD would immediately increase the newly synthesized GABA pool and thus the amount of GABA which is available for release into the synaptic cleft.

(2) AOAA is not a specific GABA-elevating drug but, besides GABA-T, inhibits a number of other enzymes in the brain with subsequent biochemical changes [42]. This might explain the pronounced pharmacological effects of AOAA observed before synaptosomal GABA levels in brain were seen to markedly increase. Accordingly, Kuriyama *et al.* [43] have concluded from their experiments with AOAA in mice that the increases in brain GABA levels and changes in seizure susceptibility after AOAA injection are completely unrelated. The present experiments suggest that the only exception from this conclusion is the anticonvulsant effect of the drug against 3-MP induced seizures. In contrast to AOAA, VPA is more specific in its GABA-elevating effect and, in addition to the increase in GABA, only causes slight alterations in taurine and aspartate levels in the brain [42, 44]. As regards aspartate levels, studies of Sarhan and Seiler [6] indicate that the alterations induced by VPA are confined to non-synaptosomal compartments of the excitatory amino acid and are thus not suspected to be related to VPA's pharmacological effects.

In conclusion, the present experiments with a newly developed synaptosomal model have provided further evidence that presynaptic increases of GABA levels by VPA may be responsible, at least in part, for the anticonvulsant effects of this drug. On the other hand, most pharmacological effects of the GABA-T inhibitor AOAA in rats seem to be unrelated to its GABA-elevating action. Experiments are under way to extend the present experimental approach also to evaluation of the effects of other well-known GABA-T inhibitors, such as γ-acetylenic GABA and γ-vinyl GABA.

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