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RELATIONSHIP BETWEEN DRUG-INDUCED INCREASES OF GABA LEVELS IN DISCRETE BRAIN AREAS AND DIFFERENT PHARMACOLOGICAL EFFECTS IN RATS

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Abstract—Following the administration of two γ-aminobutyric acid-(GABA) elevating drugs, namely aminooxyacetic acid (AOAA) and valproic acid (VPA), in rats, the relationship between the magnitude and the time course of increases in GABA levels of 11 brain regions and a number of pharmacological effects was studied. AOAA (30 mg/kg i.p.) caused significant GABA increases in all brain areas but the degree and time course of these increases showed considerable variation from region to region. The most marked effects were seen in the olfactory bulb, frontal cortex and hippocampus, in which maximum GABA elevations of 100–200% were reached 4–6 hr after AOAA injection. In all the other regions studied (corpus striatum, thalamus, hypothalamus, superior and inferior colliculus, substantia nigra, pons, medulla, cerebellum), increases in GABA were less marked and, at least in part, maximum increases (30–60% over control) were already reached by 1–2 hr. In contrast to AOAA, VPA (200 mg/kg i.p.) produced significant increases in GABA levels only in the cortex, olfactory bulb, corpus striatum, hypothalamus and cerebellum, maximum effects (15–35%) being already reached 5–30 min after VPA administration. As regards pharmacological effects, AOAA caused marked hypothermia, which was maximal by 1 hr and could be reversed by increasing ambient temperature, whereas effects of VPA on body temperature were only moderate. On the other hand, both drugs exerted an almost equal, pronounced antinociceptive effect in the hot plate test. Anticonvulsant efficacy was evaluated in three seizure models, namely the maximal (tonic extension) electroconvulsive threshold, and seizures induced by pentylenetetrazol and 3-mercaptopropionic acid. Anticonvulsant effects of AOAA against electroshock and pentylenetetrazol could only be determined 1 hr after injection, at which time AOAA was inactive against 3-mercaptopropionic acid-induced seizures. VPA proved to be clearly superior to AOAA in both anticonvulsant potency and duration of action. The marked differences in functional effects between VPA and AOAA could not be related to their differential effects on GABA levels in discrete brain regions. The data thus suggest that measurement of total GABA in brain regions without consideration of the compartmentalization of the neurotransmitter is only of limited value to use in an attempt to correlate elevation of GABA levels and pharmacological effects.

Compounds which elevate the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the brain have been widely used to study the involvement of GABA in various physiological and pathophysiological processes. Drugs which increase GABA by inhibition of the GABA-degrading enzyme, GABA aminotransferase (GABA-T; EC 2.6.1.19), have been shown to exhibit distinct pharmacological actions in rodents and other species, including anticonvulsant, antinociceptive, hypothermic and hypotensive effects (cf. refs [1, 2]). There have been repeated attempts to correlate these functional effects with the induced alterations in whole brain GABA levels. However, in view of the marked variations in GABA turnover and steady-state concentrations of different brain regions, and the fact that GABAergic neurons in different brain nuclei may play mutually counteracting roles, it is evident that the measurement of whole brain GABA cannot provide functionally interpretable information [3]. The problem of correlating drug-induced increases in GABA with pharmacological effects is further complicated by the finding that different GABA-elevating drugs may cause differential increases of regional GABA levels in the brain although the increase of whole brain levels might be similar [4, 5].

Two of the most extensively studied GABA-elevating drugs are aminooxyacetic acid (AOAA) and the anti-epileptic drug valproic acid (VPA). Although both drugs have been widely used to correlate elevation of brain GABA levels and neuropharmacological effects, especially anticonvulsant action, they differ in a number of aspects. (1) The characterization of the mechanism by which VPA increases GABA levels is considerably less complete than that of AOAA. AOAA is a potent, irreversible inhibitor of GABA-T and is thought to work by interacting with the carbonyl group of the pyridoxal phosphate cofactor of the enzyme [6]. Inhibition of GABA-T by VPA is extremely weak, but the drug is a more potent inhibitor of succinic semialdehyde dehydrogenase (SSADH: EC 1.2.1.16), the enzyme responsible for the subsequent stage in the GABA shunt pathway [7]. However, studies of Simler *et al.* [8] suggest that it is apparently not possible to raise brain GABA levels even with almost complete inhibition of SSADH. In addition to the effects on

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GABA degradation, VPA has been shown to increase the activity of the GABA-synthesizing enzyme glutamic decarboxylase (GAD; EC 4.1.1.15) [9, 10], which may participate in the action of VPA on GABA levels. (2) In rodents, anticonvulsant effects of VPA are seen with whole brain GABA increases in the range 30–60% over controls, whereas antiseizure actions of AOAA occur only with increases between 200 and 400%. Actually, although VPA is less effective than AOAA in raising whole brain GABA levels, VPA is equally or even more effective than AOAA in raising GABA levels in certain discrete brain regions [5]. (3) With VPA, peak anticonvulsant activity is reached within 5 min following i.p. injection [11], but it is not known if GABA levels are already elevated after this short time interval. After AOAA, peak activity occurs within 1–6 hr, depending on the species and the seizure test used. Although in some studies on AOAA no temporal correlation between anticonvulsant effects and brain GABA increases was found (e.g. ref. [12]), others demonstrated a close correlation (e.g. ref. [13]).

In the present study, we investigated the temporal relationship between VPA and AOAA-induced increases in regional GABA levels and a number of pharmacological effects of both drugs in rats. The rationale for using AOAA and VPA for this study was two-fold. First, although both drugs are known to cause differential effects on regional GABA levels, the temporal correlation of the respective alterations with pharmacological effects has not yet been studied. Thus, this study may be one approach to sort out functional effects of increased GABA levels in particular brain areas. Secondly, the experiments may eventually help to explain some of the differences between VPA and AOAA discussed above.

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 GmbH (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 dissolved in 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 Versuchstierzucht, Borchen, F.R.G.) weighing 200–220 g. Rats were kept in groups of ten in Makrolon® cages at constant temperature (25°) and controlled humidity (*ca.* 50%) with a 12 hr light cycle beginning at 7 a.m., and were fed Altromin® 1324 standard food (Altromin, Lage, F.R.G.). Unless indicated otherwise, all experiments were carried out at 25°.

Determination of anticonvulsant activity. The threshold for maximal (tonic extension) electroconvulsions was determined in groups of 15 rats at different times after administration of AOAA (30 mg/kg i.p. or s.c.) and VPA (200 mg/kg i.p.), respectively. Electroshock was applied by eye electrodes using a Lafayette A-615 B shocker (Lafayette Instrument Co., IN). Stimulation data were 50 Hz for 0.2 sec with the serial-resistance of the apparatus set to 10 kΩ. The extension of the hindlimbs was taken as the end-point. The threshold was determined by the 'up and down' method of Kimball *et al.* [14] 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 ten rats, pentylenetetrazol (70 mg/kg s.c.) was administered at different times after AOAA (10 and 30 mg/kg i.p.) and VPA (200 mg/kg i.p.), respectively. Following injection of pentylenetetrazol, animals were observed for 30 min for the presence or absence of seizures and the following scale was used to rate the severity of the convulsions: 0, no seizure; 0.5, clonic seizure; 1, clonic–tonic seizure with loss of righting reflexes. Intermittent twitching was generally disregarded. The scores of the ten animals were added up and used for comparison between control groups and experimental groups.

In further groups of ten rats, 3-mercaptopropionic acid (3-MP; 50 mg/kg i.p.) was administered 1 hr after AOAA (30 and 50 mg/kg i.p.) or 0.25 hr after VPA (200 mg/kg i.p.), respectively. The animals were observed during the following 30 min for the occurrence of seizures and seizure response was rated as follows: 0, no seizure; 1, running seizures; 2, clonic seizures; 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 ten 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' of temperature 56°. The time in sec 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). The experiments with AOAA were repeated in rats which received the drug at an ambient temperature of 30–33°.

GABA determinations in brain regions. Regional GABA concentrations in the brain were determined in groups of five rats at different times after i.p. injection of AOAA (30 mg/kg) and VPA (200 mg/kg). Rats were killed by decapitation, the brains were rapidly removed and dissected on a cold plate at −18° (Leitz Kryomat, Wetzlar, F.R.G.) into 11 brain regions within 4 min after decapitation. The individual regions were rapidly weighed and homogenized in 2 ml of 80% ethanol (tubes immersed in a bath of methanol at −30°). After centrifugation at 4000 rpm for 10 min at −5°, the pellet was resuspended in 1 ml of 80% ethanol and again centrifuged. The two supernatants were combined and evaporated to dryness by a stream of nitrogen. The residue was dissolved in 0.5 ml of pyrosphosphate buffer (0.1 M, pH 8.3) and GABA was measured in aliquots of 0.2 ml by the enzymatic 'GABAase' method as

described by Baxter [15]. Protein content of each brain region was determined by the method of Lowry *et al.* [16] as modified by Markwell *et al.* [17]. GABA concentrations in each treated group of rats were compared with those in a control group of five animals which were killed immediately prior to the treated animals.

To examine if post-mortem increase of GABA occurred during the dissection, five animals were killed by decapitation 2.5 min after injection of 3-MP (100 mg/kg i.p.; see ref. [18]) and GABA levels were compared with those determined in a control group decapitated at the same time without 3-MP pretreatment.

Statistics. Arithmetical means and S.E. are given for biochemical and pharmacological 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 the control group of the same day by the unpaired Student's *t*-test.

RESULTS

Control GABA levels in rat brain regions

The control GABA levels (means ± S.E. of 135 rats) in μmole/g wet tissue (nmole/mg protein) were: olfactory bulb, 3.36 ± 0.09 (27 ± 0.81); frontal cerebral cortex, 1.21 ± 0.04 (9.8 ± 0.37); corpus striatum, 2.13 ± 0.06 (16.5 ± 0.46); hippocampus 1.65 ± 0.06 (12.4 ± 0.46); thalamus, 3.44 ± 0.13 (24.3 ± 0.71); hypothalamus, 3.68 ± 0.12 (25.1 ± 0.71); superior and inferior colliculus, 2.23 ± 0.06 (20.1 ± 0.59); substantia nigra, 2.54 ± 0.1 (19.9 ± 0.17); pons, 0.98 ± 0.03 (8.35 ± 0.25); medulla, 0.91 ± 0.04 (7.7 ± 0.24); and cerebellum, 0.84 ± 0.02 (7.9 ± 0.27). In the experiment in which rats were given 3-MP (100 mg/kg i.p.) 2.5 min prior to decapitation, the GABA levels did not differ from those determined without 3-MP pretreatment (Fig. 1).

Effect of AOAA and VPA on regional GABA levels

Following administration of AOAA (30 mg/kg i.p.), the GABA levels significantly increased in all regions studied (Fig. 2). However, the time course of this effect and the per cent increases of GABA levels over control showed considerable variation from region to region. The most marked effects were seen in the frontal cortex and hippocampus, in which maximum GABA elevations of *ca* 200% were reached 6 hr after AOAA injection. In the olfactory bulb, a maximum GABA increase of 100% occurred by 4 hr. In all other regions, GABA increases were less marked and, at least in part, maximum increases (30–60% over control) were already reached between 1 and 2 hr after injection of AOAA. Twelve hr following AOAA treatment, GABA levels had returned to control values in all regions except the cortex and hippocampus.

VPA (200 mg/kg i.p.) produced moderate increases in regional GABA levels which achieved significance only in the cortex, olfactory bulb, corpus striatum, hypothalamus and cerebellum (15–35% over control; Fig. 3). The elevation of GABA levels was rapid in onset, maximum effects being reached 5–30 min after VPA injection. If one compares the profile or regional GABA increases obtained with AOAA and VPA, the differences were most marked in the cerebral cortex, hippocampus and olfactory bulbs, in which the effect of AOAA was considerably larger than that determined for VPA. In other

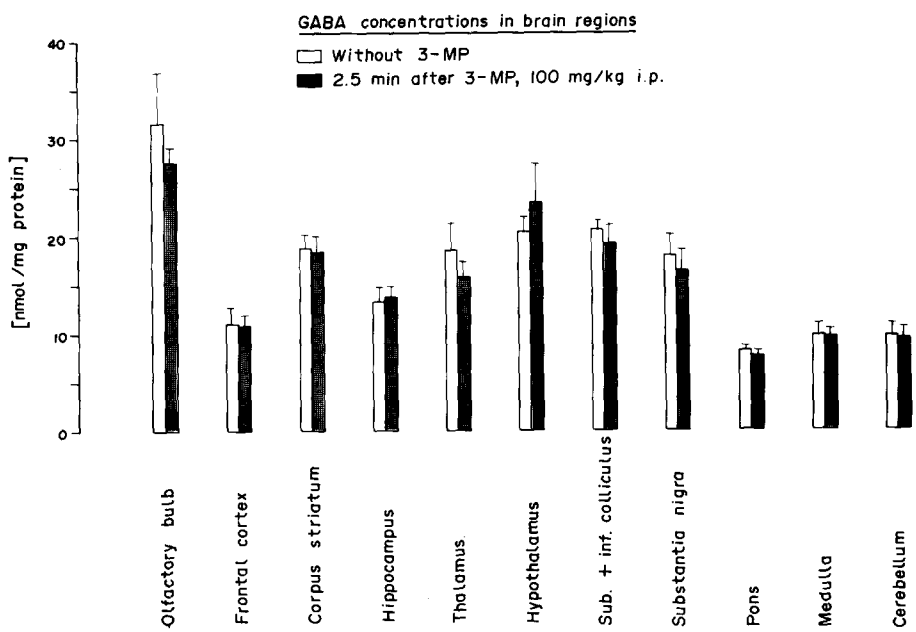

Fig. 1. Regional GABA levels in rat brain determined after decapitation and rapid dissection at −18°. To control for post-mortem increases at the conditions of dissection, rats were either decapitated 2.5 min after injection of saline or 2.5 min after treatment with 3.MP (100 mg/kg i.p.). Values are mean ± S.E. of five rats.

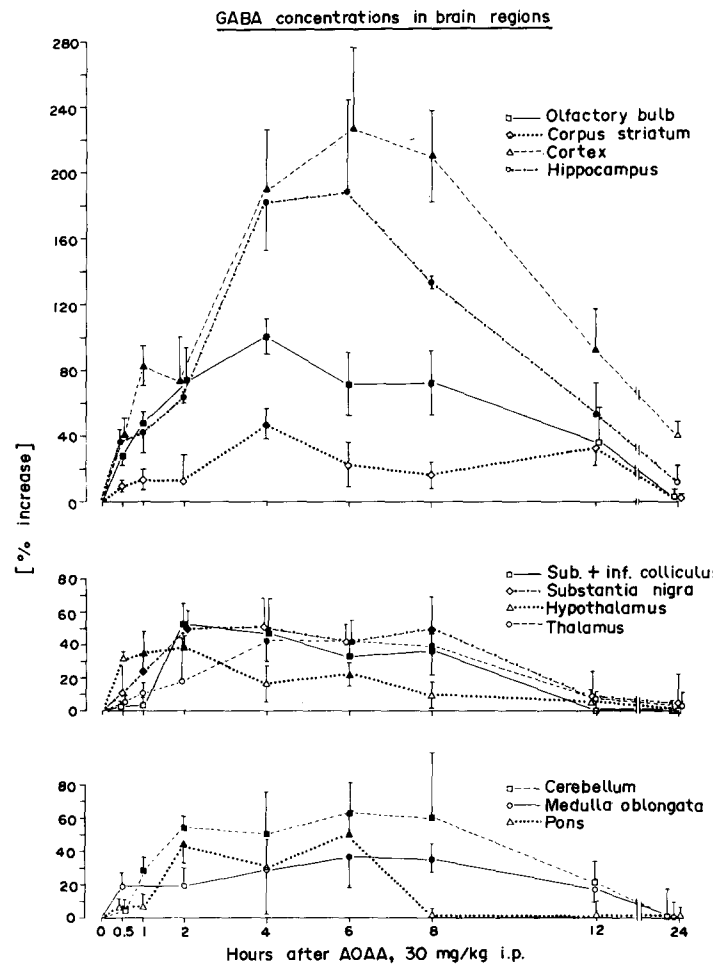

Fig. 2. Effect of AOAA (30 mg/kg i.p.) on regional GABA levels in rat brain. Results are expressed as per cent increase (mean ± S.E. of five rats) over concurrent control determinations (see text for absolute control values). Significance of differences ($P < 0.05$) to the individual controls is indicated by filled symbols.

regions, the differences in maximum GABA increases between the two drug treatments were less marked, and in some regions, e.g. the hypothalamus, both drugs exerted essentially the same effect.

Functional effects of AOAA and VPA

Following i.p. injection of AOAA (30 mg/kg), there was a pronounced decrease in rectal temperature, which was maximal at 1 hr (Fig. 4). Body temperature then rapidly returned to the control value and no significant alteration was observed at 4, 6, 8 and 12 hr. In the hot plate test, AOAA exerted a very marked antinociceptive effect with a time course similar to that of the induced hypothermia. When ambient temperature was increased to 30–33°, the hypothermic effect of AOAA was counteracted but the antinociceptive effect became more pronounced (Fig. 4). The anticonvulsant efficacy of AOAA in rats was determined in three different seizure models, namely the maximal electroshock seizure threshold and seizures induced by pentylenetetrazol and 3-MP. As shown in Fig. 4, a significant increase in the electroconvulsive threshold was observed 1 hr following AOAA (30 mg/kg i.p.) but thereafter the threshold declined even below control values up to 8 hr. A similar time course was determined in terms of the effect on pentylenetetrazol-induced seizures, which were attenuated only at 1 hr after i.p. injection of 30 mg/kg AOAA (Fig. 4). A lower dose (10 mg/kg i.p.) was without any effect on pentylenetetrazol seizures (not illustrated). Subcutaneous administration of AOAA (30 mg/kg) exerted no effect on the electroconvulsive threshold (determined at 1 and 4 hr after AOAA injection). Furthermore, 1 hr post-injection, AOAA (30 or 50 mg/kg i.p.) had no significant effect on seizures induced by 3-MP but increased the percentage of animals dying by the convulsant drug from 30 to 90–100% (Table 1).

In contrast to AOAA, VPA (200 mg/kg i.p.) exerted a biphasic effect on rectal temperature (Fig. 5). A short-lasting increase was observed by 0.5 hr, whereas at 2, 4, 6, and 8 hr a moderate but significant decline in body temperature was determined. With respect to the hot plate test, VPA displayed an antinociceptive effect which was more rapid in onset and shorter-lasting compared to AOAA. The maxi-

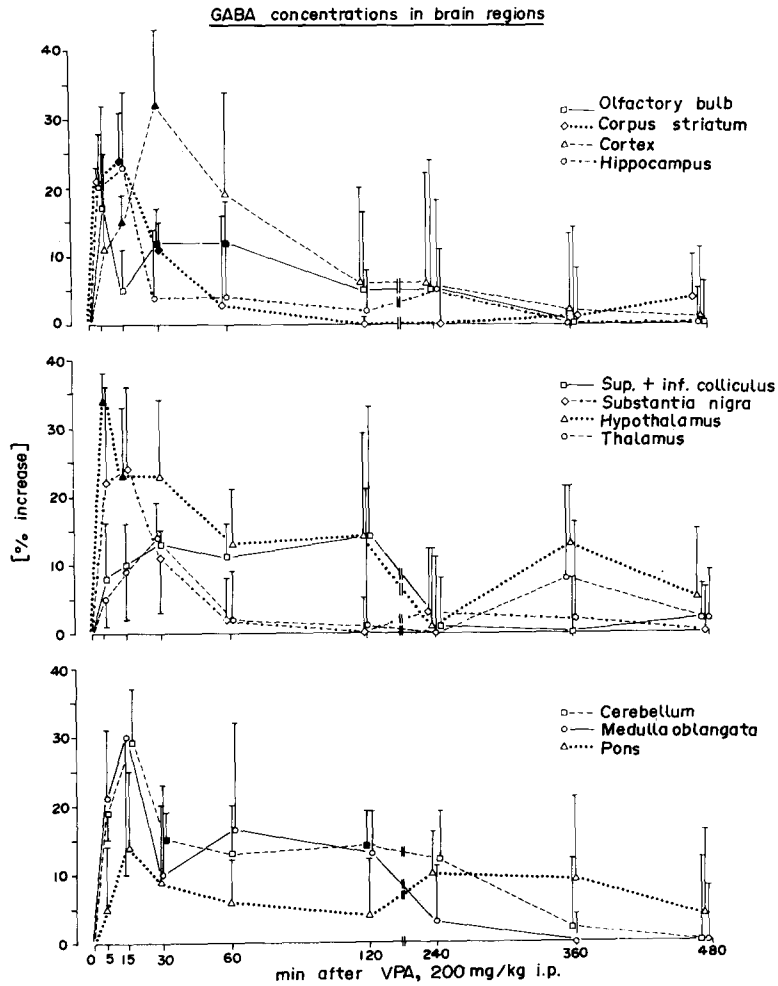

Fig. 3. Effect of VPA (200 mg/kg i.p.) on regional GABA levels in rat brain. Results are expressed as per cent increase (mean ± S.E. of five rats) over concurrent control determinations (see text for absolute control values). Significance of differences ($P < 0.05$) to the individual controls is indicated by filled symbols.

mum 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 more potent than AOAA against both electroshock and pentylenetetrazol-induced seizures (Fig. 5). Peak anticonvulsant activity occurred 5–15 min after VPA administration and a significant increase in seizure threshold was determined up to 8 hr. This difference in anticonvulsant potency to AOAA was even more pronounced in terms of 3-MP-induced seizures, as VPA provided complete protection 0.25 hr following i.p. administration of 200 mg/kg (Table 1).

DISCUSSION

The main purpose of this study was to determine whether different functional effects of GABA-elev-

Table 1. Effect of AOAA and VPA on seizures induced by 3-MP (50 mg/kg i.p.)

Drug	Time (hr)	Dose (mg/kg i.p.)	No. of rats	Seizure severity score	Death (%)
Control			20	4.1 ± 0.34	30
AOAA	1	30	10	4.3 ± 0.52	90
AOAA	1	50	10	3.3 ± 0.56	100
VPA	0.25	200	10	0.2 ± 0.2*	0

The seizure severity score represents the arithmetic mean (±S.E.) of the maximum individual responses for each animal in the group. Significant differences between concurrent control and drug-treated groups are denoted by * ($P < 0.001$).

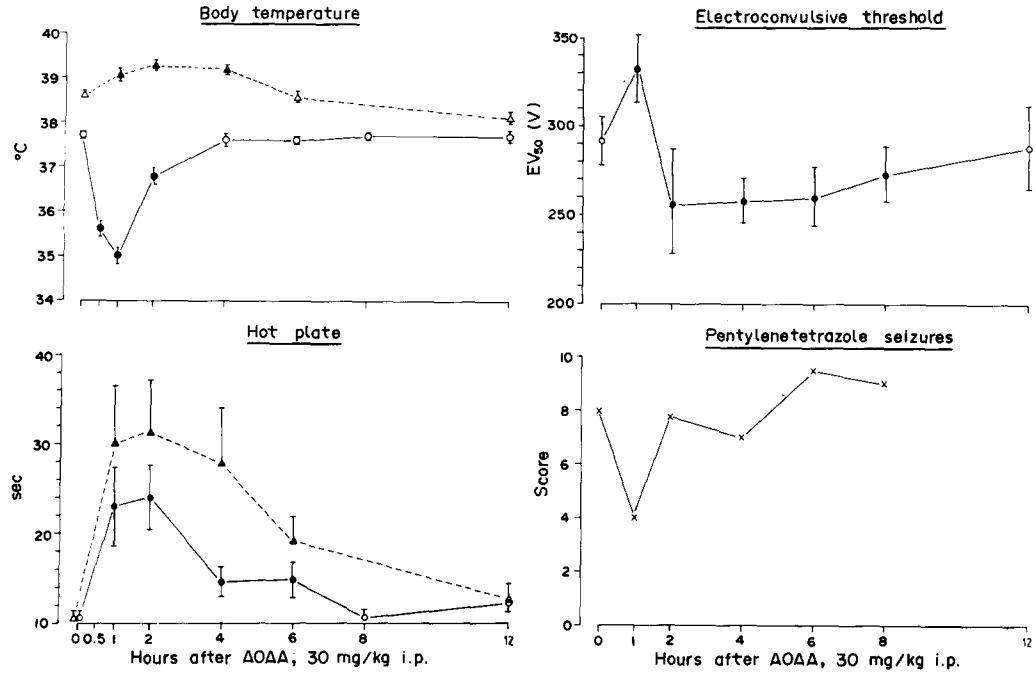

Fig. 4. Effects of AOAA (30 mg/kg i.p.) on rectal temperature, pain response and seizure behaviour in rats. Body temperature and reaction time (in sec) in the hot plate test were determined at ambient temperature of 25° (solid lines) and 30–33° (dashed lines). At both temperatures, saline-injected controls remained within two standard deviations of the initial values throughout the course of the experiment. Ten animals were used per time point and results are given as mean ± S.E. Significance of differences ($P < 0.05$) is indicated by filled symbols. The effects of AOAA on the threshold for maximal (tonic extension) electroconvulsions and on seizures induced by pentylenetetrazol (70 mg/kg s.c.) were determined at an ambient temperature of 25°. The electroconvulsive threshold is given as the voltage inducing an extension of the hindlimbs in 50% of the rats (EV_{50}). The vertical bars represent the confidence limits for 95% probability; 15 rats were used for each determination. Significance of difference ($P < 0.05$) is indicated by filled symbols. The values shown for pentylenetetrazol were derived from the scale used to rate the severity of the seizures (see Materials and Methods). Each value was determined in ten rats.

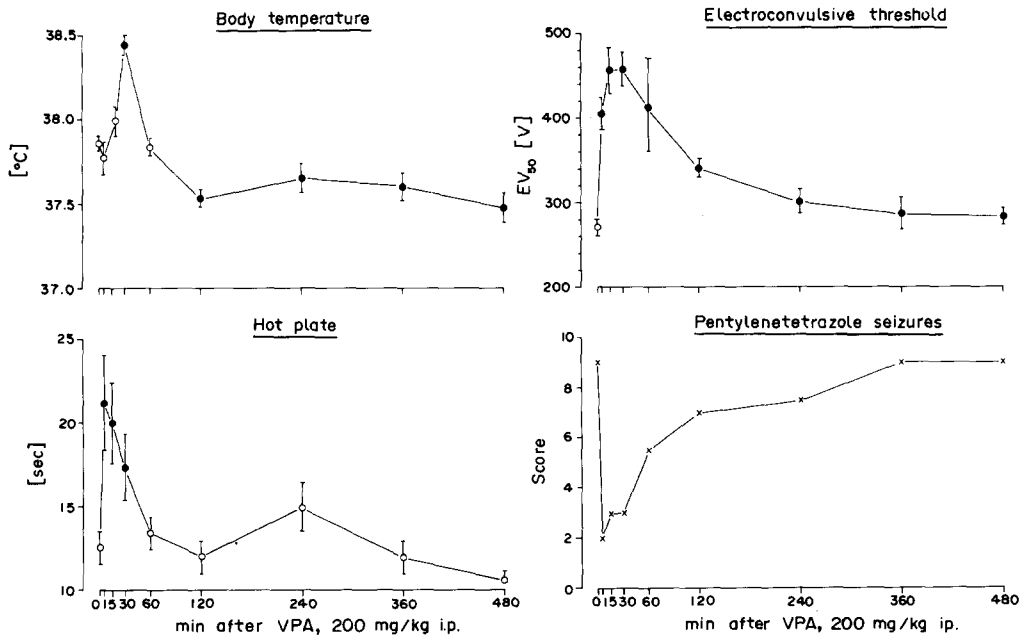

Fig. 5. Effects of VPA (200 mg/kg i.p.) on rectal temperature, pain response and seizure behaviour in rats. All experiments were carried out at an ambient temperature of 25°. For further explanation see legend to Fig. 4.

ating drugs can be related to the differential effects of such compounds on GABA levels in discrete brain areas. First of all, a control experiment was carried out to examine if any post-mortem increase of GABA occurred during the dissection of brain areas, which might bias results obtained with the GABA-elevating drugs to be studied. The finding that GABA levels in rats pretreated with 3-MP, which had been shown previously to prevent post-mortem increase of GABA completely [18, 19], did not differ from those determined without 3-MP pretreatment strongly indicated that post-mortem increases did not occur during rapid dissection of the brain at −18°. This was also indicated by the similarity of the present regional GABA levels with those determined by Balcom *et al.* [20] in the same rat brain regions after microwave fixation. The only significant difference was that GABA levels measured in the substantia nigra were 30% lower in the present study compared to the data of Balcom *et al.* [20], which may be attributed to differences in the dissection technique, e.g. contamination with surrounding mesencephalic tissue [19].

The profile of GABA increases obtained with AOAA and VPA is in general agreement with that reported by Iadarola *et al.* [5] for six brain regions of the rat, although no time courses were determined by these authors. With both drugs, the magnitude of the change in regional GABA levels was obviously not a function of the normal steady-state GABA content. This suggests that the differences in GABA increases across areas could be due to differential drug distribution in the brain. Actually, whereas no information is available on AOAA distribution in the brain, recent studies on VPA indicated that its concentrations in different brain regions are not uniform [21].

With respect to functional effects, AOAA and VPA differed in a number of aspects. As reported previously [13], AOAA produced a marked hypothermia which could be reversed by increasing the ambient temperature. This reversal has already been demonstrated for other GABA-T inhibitors and is consistent with an inhibitory function for GABA in thermoregulation [22]. The pronounced hypothermic effect of AOAA could thus possibly be related to the increase of GABA in hypothalamus, the brain region considered to play the major role in thermoregulation. However, unlike AOAA, VPA exerted only moderate effects on body temperature, although the hypothalamic GABA increase brought about by VPA was equal to that seen after AOAA.

In recent years, a role of GABA in the mediation of pain and antinociceptive drug action has repeatedly been discussed (cf. ref. [23]). Drugs which increase the neuronal function of GABA, especially GABA receptor agonists, have been shown to decrease the pain response in different test models and enhance the action of opiates such as morphine [23]. Antinociceptive action has also been reported for VPA [24] and AOAA [25] in mice. The present experiments in rats demonstrate that at the doses employed the antinociceptive potency of VPA is of the same order of magnitude as that of AOAA. Although there are multiple sites in the brain involved in the perception of pain and the modulation of nociceptive reflexes, the consistent antinociceptive action of VPA and AOAA could possibly relate to the almost equal effect of both drug treatments on GABA levels in the hypothalamus, which is thought to play an important role in mediating antinociceptive drug action [26].

As regards anticonvulsant action, VPA proved clearly superior to AOAA in both potency and duration of action. With 30 mg/kg AOAA i.p., an anticonvulsant effect against seizures induced by electroshock or pentylenetetrazol could only be demonstrated 1 hr after administration. At this time, AOAA at both 30 and 50 mg/kg was without significant effect on seizures induced by 3-MP, an inhibitor of the GABA-synthesizing enzyme GAD, which is thought to cause convulsions by the induced decrease of GABA in nerve terminals [27, 28]. AOAA not only failed to protect animals against 3-MP-induced seizures but increased the mortality rate from 30 to 90–100%. This may well be due to additive inhibition of GAD by AOAA and 3-MP since AOAA, at least *in vitro*, is a potent inhibitor of GAD [13]. Higher doses of AOAA were not evaluated because marked general sedation and toxicity are encountered in rats with doses above 50 mg/kg [29, 30]. The weak and short-lasting anticonvulsant effect of AOAA determined in rats is in contrast to previous data reported for mice, in which 20–30 mg/kg AOAA proved to be highly potent in suppressing seizures induced by electroshock, pentylenetetrazol and 3-MP [13, 25, 28, 31]. Although in these recent experiments in mice, s.c.-administered AOAA was more potent than treatment by the i.p. route [25], the opposite was the case in rats. The species-related differences in anticonvulsant potency of AOAA are even more evident in gerbils, in which reflex seizures can be suppressed with an ED_{50} of less than 1 mg/kg i.p. [32].

The elevation in regional GABA concentrations determined in rat brain after AOAA injection did not parallel the time course of effect on electroshock and pentylenetetrazol-induced seizures. Actually, maximum GABA increases were reached in most regions after 2–6 hr, at which time no seizure protection was determined. A similar temporal dissociation between antiseizure activity of AOAA and increased (whole brain) GABA levels had been found by Kuriyama *et al.* [12] in mice. However, subsequent studies, also using mice, determined a close temporal correlation of GABA increases with anticonvulsant activity of AOAA, the maximal effect being after 6 hr [13].

In contrast to the apparent discrepancies surrounding AOAA's time course of anticonvulsant activity, a very close correlation has repeatedly been observed in mice between the time course of whole brain GABA increases and seizure protection after VPA in all experiments that have examined the two variables [33–37]. Accordingly, the present experiments with VPA in rats demonstrate that both anticonvulsant activity and regional GABA increases are maximal after 5–30 min following administration, and thereafter both variables decline rapidly towards control values.

The striking difference in anticonvulsant activity between VPA and AOAA could not be related to

differential effects of both drugs on regional GABA levels. In this respect it should be noted that AOAA is not a specific GABA-elevating agent but, besides GABA-T, inhibits a number of other enzymes in the brain with subsequent biochemical changes [38]. Thus, part of the pharmacological effects of AOAA may be unrelated to GABA metabolism. This possibility might also apply, although to a lesser extent, to VPA which, for instance, has been shown to decrease brain aspartate levels in mice [39]. On the other hand, if the change in GABA content is the common mechanism by which these drugs exert their anticonvulsant effect, then the precise nature of their interaction with the GABA system must differ in some important aspect. Previous experiments by Gale and coworkers, using the technique of the GABA-denervated substantia nigra in rats, have suggested that VPA preferentially increases the GABA concentration in nerve terminals while AOAA exerts its preponderant effect on GABA levels in glial cells and neuronal perikarya (cf. ref. [3]). Thus, the marked changes in GABA levels of brain regions brought about by AOAA in rats need not necessarily reflect alterations relevant to synaptic transmission, which could explain the weak anticonvulsant effect of this drug. Differences in the compartmentalization of GABA increases may also help to explain the controversial results reported for AOAA in different rodent species.

In conclusion, based on the present observations, we propose that the measurement of total GABA in discrete brain regions without compartmental analysis is not an appropriate parameter to use in an attempt to correlate elevation of GABA levels and neurophysiological effects such as anticonvulsant action. Although the direct measurement of the GABA content of nerve terminals in different brain regions is beyond the scope of current technology, we intend to determine by means of subcellular fractionation studies whether the magnitude and time course of the functional effects of VPA and AOAA can be correlated with alterations of synaptosomal GABA levels of discrete brain areas.

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REFERENCES

1. P. J. Schechter and Y. Tranier, in *Enzyme-Activated Irreversible Inhibitors* (Eds. N. Seiler, M. J. Jung and J. Koch-Weser), p. 149. Elsevier, Amsterdam (1978).
2. W. Löscher, in *Neurotransmitters, Seizures, and Epilepsy* (Eds. P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum and E. H. Reynolds), p. 93. Raven Press, New York (1981).
3. M. J. Iadarola and K. Gale, *Molec. cell. Biochem.* **39**, 305 (1981).
4. M. J. Jung, in *Enzyme-Activated Irreversible Inhibitors* (Eds. N. Seiler, M. J. Jung and J. Koch-Weser), p. 135. Elsevier, Amsterdam (1978).
5. M. J. Iadarola, A. Raines and K. Gale, *J. Neurochem.* **33**, 1119 (1979).
6. D. P. Wallach, *Biochem. Pharmac.* **5**, 323 (1961).
7. P. K. P. Harvey, H. F. Bradford and A. N. Davison, *FEBS Lett.* **52**, 251 (1975).
8. S. Simler, L. Ciesielski, M. Klein, S. Gobaille and P. Mandel, *C.R. Soc. Biol.* **175**, 114 (1981).
9. W. Löscher, *Biochem. Pharmac.* **30**, 1364 (1981).
10. N. I. Phillips and L. J. Fowler, *Biochem. Pharmac.* **31**, 2257 (1982).
11. R. W. Kerwin, H.-R. Olpe and M. Schmutz, *Br. J. Pharmac.* **71**, 545 (1980).
12. K. Kuriyama, E. Roberts and M. K. Rubinstein, *Biochem. Pharmac.* **15**, 221 (1966).
13. W. Löscher and H.-H. Frey, *Biochem. Pharmac.* **27**, 103 (1978).
14. A. W. Kimball, W. T. Burnett and D. G. Doherty, *Radiation Res.* **7**, 1 (1957).
15. C. F. Baxter, in *Methods of Neurochemistry* (Ed. R. Fried), p. 1. Marcel Dekker, New York (1972).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. M. A. K. Markwell, S. M. Haas, L. L. Bieber and N. E. Tolbert, *Analyt. Biochem.* **87**, 206 (1978).
18. E. Carmona, C. Gomes and G. Trolin, *Naunyn-Schmiedeberg's Archs Pharmac.* **313**, 221 (1980).
19. J. A. M. van der Heyden and J. Korf, *J. Neurochem.* **31**, 197 (1978).
20. G. J. Balcom, R. H. Lenox and J. L. Meyerhoff, *J. Neurochem.* **24**, 609 (1975).
21. E. Mesdjian, L. Ciesielski, M. Valli, B. Bruguerolle, G. Jadot, P. Bouyard and P. Mandel, *Prog. Neuro-Psychopharmac. Biol. Psychiat.* **6**, 223 (1982).
22. P. J. Schechter and Y. Tranier, *Pharmac. biochem. Behav.* **6**, 227 (1977).
23. F. V. DeFeudis, *Trends pharmac. Sci.* **3**, 444 (1982).
24. K. Shuto and T. Nishigaki, *Pharmacometrics* **4**, 937 (1970).
25. W. Löscher, *Naunyn-Schmiedeberg's Archs Pharmac.* **315**, 119 (1980).
26. T. S. Dubas and J. M. Parker, *Archs int. Pharmacodyn.* **194**, 117 (1971).
27. A. Karlsson, F. Fonnum, D. Malthe-Sørensen and J. Storm-Mathisen, *Biochem. Pharmac.* **23**, 3053 (1974).
28. W. Löscher, *Biochem. Pharmac.* **28**, 1397 (1979).
29. J. P. DaVanzo, M. E. Greig and M. A. Cronin, *Am. J. Physiol.* **201**, 833 (1961).
30. J. R. Walters, N. Eng, D. Pericic and L. P. Miller, *J. Neurochem.* **30**, 759 (1978).
31. W. E. Stone, *Epilepsia* **18**, 507 (1977).
32. W. Löscher, H.-H. Frey, R. Reiche and D. Schultz, *J. Pharmac. exp. Ther.* **226**, 839 (1983).
33. S. Simler, L. Ciesielski, M. Maitre, H. Randrianarisoa and P. Mandel, *Biochem. Pharmac.* **22**, 1701 (1973).
34. P. J. Schechter, Y. Tranier and J. Grove, *J. Neurochem.* **31**, 1325 (1978).
35. W. D. Lust, H. J. Kupferberg, J. V. Passonneau and J. K. Penry, in *Clinical and Pharmacological Aspects of Sodium Valproate (Epilim) in the Treatment of Epilepsy* (Ed. N. J. Legg), p. 123. MCS Consultants, Tunbridge Wells (1976).
36. J. Y. Lacolle, B. Ferrandes and P. Eymard, in *Advances in Epileptology. Psychology, Pharmacotherapy and New Diagnostic Approaches* (Eds. H. Meinardi and A. J. Rowan), p. 162. Swets & Zeitlinger, Amsterdam (1978).
37. W. Löscher, *Neurochem. int.* **3**, 397 (1981).
38. T. L. Perry and S. Hansen, *J. Neurochem.* **30**, 679 (1978).
39. P. J. Schechter, Y. Tranier and J. Grove, *J. Neurochem.* **31**, 1325 (1978).