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EFFECTS OF INHIBITORS OF GABA-TRANSAMINASE ON HOLE-BOARD EXPLORATION AND ON TEMPERATURE

RELATION WITH EFFECTS ON QUASI-MORPHINE ABSTINENCE BEHAVIOUR INDUCED BY SODIUM DIPROPYLACETATE

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Abstract—Four inhibitors of γ-aminobutyric acid transaminase (GABA-T) were investigated together with respect to their effects on hole-board exploration and temperature and the relation with effects on quasi-morphine-abstinence behaviour induced by dipropylacetate (DPA) in rats.

Amino-oxyacetic acid (AOAA), γ-acetylenic-GABA (GAG), γ-vinyl-GABA (GVC) and ethanolamine-*O*-sulfate (EOS) were found to reduce hole-board exploration especially in the higher doses used, although the time-course of the effect was different for the compounds. For EOS and GVG the decrease in hole-board exploration paralleled a strong hypothermic effect. The compounds AOAA and GAG exerted a less and more transient hypothermic effect. However, the decrease in hole-board exploration did not fall in with this decrease in temperature.

AOAA and GAG were found to decrease DPA-induced body shakes and locomotor activity, while GVG and EOS had no effect on body shakes and transient effects but opposite to each other, on locomotor activity.

The efficacy of the GABA-T-inhibitors was measured biochemically, and the influence on the activity of glutamate decarboxylase (GAD) was also determined. AOAA and GAG were found to be strong inhibitors of GABA-T whereas the other two compounds were less efficient in the used doses. In addition AOAA and GAG influenced the activity of GAD strongly, while using GVG only a small decrease was found. The results suggest that the anti-quasi-withdrawal, the sedative and the hypothermic effects are not related to each other nor related to an effect on GABA-T. The suppressive effects on quasi-withdrawal body shakes, however, could be related to the inhibition of GAD and a hypothesis involving a compartmentalized action of DPA on GABA-metabolism has been proposed.

Administration of sodium-dipropylacetate (valproate, DPA)† into rats has been shown to induce a syndrome consisting of "wet dog" shakes and enhanced locomotor activity resembling morphine abstinence behaviour [1, 2]. Similar effects could be obtained using analogs of DPA and a relation with the inhibition of degradation of γ-aminobutyric acid (GABA) appeared to exist [3]. Since this behaviour could be antagonized by GABA-antagonists picrotoxin and bicuculline, the enhancement of the GABA-concentration by DPA, inhibiting the succinic semialdehyde dehydrogenase (EC 1.1.1.16), has been thought to be responsible for the induction [2, 4].

From the mechanistic point of view the suppressive effect of the inhibitor of GABA-transaminase (EC 2.6.1.19), amino-oxyacetic acid (AOAA) [2], on body shakes induced by DPA was difficult to understand and gave rise to a hypothesis involving a different cellular localization of the effects of AOAA and DPA. DPA is supposed to act in nerve terminals specifically, whereas AOAA should act in the remaining areas viz. neuronal bodies and possibly glial cells [2, 5].

The effects of several other GABA-T inhibitors, viz. gamma-acetylenic (GAG), gamma-vinyl GABA (GVG) and ethanolamine-O-sulfate (EOS), have been investigated in this study. AOAA has been studied also for comparison. An important difference between these compounds is their effect on glutamate decarboxylase (GAD) (EC 4.11.15), consequently GABA-T and GAD-activities were also measured.

From the functional point of view, effects on locomotor activity and on body shakes may be related to a sedative and a hypothermic action, respectively. Therefore the effects on the hole-board exploration and on temperature have been studied using the same dosages and pretreatment time.

MATERIALS AND METHODS

Animals

Male albino rats (100–200 g) randomly selected from a random bred Wistar strain (CPB-TNO, Zeist,

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† Abbreviations used: GABA, γ-aminobutyric acid; GABA-T, GABA-transaminase; GAD, glutamate decarboxylase; AOAA, amino-oxyacetic acid; GVG, γ-vinyl GABA; GAG, γ-acetylenic GABA; EOS, ethanolamine-*O*-sulfate; DPA, sodium dipropylacetate.

The Netherlands) were used in all experiments. The animals were housed six to nine in a wire cage with food and water *ad libitum*. Lights were kept on from 6.00 a.m. till 6.00 p.m. unless otherwise stated. The experiments were performed between 9.00 a.m. and 4.00 p.m. in a room with white noise and a temperature of 22–24°.

Chemicals and drugs

Chemicals. NAD and NADH were purchased from Boehringer Mannheim. GABA, L-Glutamate, pyridoxal-5-phosphate (PLP) and sodium thiosulfite were obtained from Sigma Chemical Company; 2-oxoglutarate, 2-mercaptoethanol and Triton X-100 were purchased from BDH, orthophtalaldehyde from Serva and CM-sepharose 6B from Pharmacia. Buffer constituents and other reagents were obtained from Merck (Darmstadt).

Drugs. Amino-oxyacetic (AOAA, Sigma), gamma-acetylenic GABA (GAG) and gamma-vinyl GABA (GVG, both from Merrell, Strasbourg) were dissolved in saline. Ethanolamine-*O*-sulfate (EOS, Fluka) was purified by dissolving in distilled water and precipitating by absolute ethanol as described by Leach and Walker [6]. Before administration EOS was dissolved in distilled water. Sodium dipropylacetate (Albic, Maassluis) was dissolved in water (100 mg/ml). All compounds were administered intraperitoneally except for EOS which was given subcutaneously.

Temperature measurement and hole-board exploration

Body temperature was measured with a thermistor probe inserted 3–4 cm into the rectum and recorded by a potentiometer (Ellab). Animals were housed with a reversed day–night scheme (lights on from 6.00 p.m. till 6.00 a.m. for at least 14 days before the start of the experiment and were deprived of food during the last 24 hours. Temperature was recorded 1 hr before and 2.5 hr or 5 hr after administration. Hole-board exploration was measured in the same time using these animals. The hole-board consists of a PVC square (40 × 40 cm) with 23 holes (2 cm dia.). Animals were used only once in the hole-board and were tested 2.5 or 5 hr after injection of a drug during 5 min. These periods fell directly after temperature recording. After every test the hole-board was cleaned using tap-water. For each compound 6 animals per group were used.

Quasi-morphine withdrawal behaviour

In separate experiments, rats were placed in Perspex cages with sawdust bedding and were allowed to habituate for at least 20 min whereafter DPA (300 mg/kg) was administered intraperitoneally. Behavioural observation was started directly after injection and lasted for 15 min. Although a whole scale of symptoms can be observed after injection of DPA [6], only body shakes were counted.

Locomotor activity was measured concurrently also for 15 min using a photocell activity meter with one beam. In the experiment with GAG, locomotor activity was scored manually. This figure therefore, was somewhat lower than in the other experiments because the light beam registered individual leg and tail movements in many cases. However, this fact did not result in a qualitative difference in the effects of the compounds. For each compound 6 animals per group were used.

Determination of brain GABA-T and GAD activities

Rats were sacrificed by decapitation and the brains were removed rapidly and dissected on ice. Cortex tissue was obtained by making a 2 mm thick vertical slice at the level of the neostriatum. The left and right parts of cortex and of the cerebellum were frozen apart on dry ice and used for GABA-T or GAD measurement, respectively. For each compound 5 animals per group were used.

GABA-T assay. For the GABA-T assay a coupled enzyme system was used in which GABA is converted into succinic-semialdehyde (SSA) by transamination and subsequently oxidized to succinic acid by endogenous SSA-DH in the presence of NAD [7]. Tissue was homogenized in a 10-fold quantity of 0.32 M sucrose solution, using a Branson sonifier. One volume of the homogenate was added to 3 vol. of ice-cold Triton medium (0.67% w/v Triton X-100, 50 mM Tris–HCl. pH 8.5 and 4.5 mM 2-mercaptoethanol) and kept on ice water for 1 hr before further incubation. Incubation with substrate was carried out at 37° for 1 hr. The solution was prepared as follows: 50 µl homogenate, 20 µl 50 mM-GABA in 50 mM Tris–HCl pH 8.5 and 50 µl Tris buffer pH 8.5 with 1 mM 2-oxoglutarate, 2.4 mM NAD^+ and 5 mM 2-mercaptoethanol. The reaction was started by placing the tubes in a waterbath on 37° and was stopped by placing the tubes in ice water. Afterwards NADH formation was measured as described by De Boer and Bruinvels [7].

GAD assay. For the assay GABA was synthesized from L-glutamate and was separated from this compound by ion exchange chromatography. The amino-acid was determined measuring fluorescence after reaction with *O*-phtalaldehyde in the presence of 2-mercaptoethanol. Tissue was homogenized in 0.32 M sucrose solution, containing 0.05% (w/v) $Na_2S_2O_3$. One volume of the homogenate was added to 3 vol of ice-cold Triton medium (0.67% w/v Triton X-100, 50 mM K_2HPO_4/KH_2PO_4, pH 6.5 and 0.05% $Na_2S_2O_3$) and kept on ice water for 1 hr before further incubation. Incubation with substrate was carried out at 38° for 1 hr. 500 µl homogenate was added to 500 µl incubation mix containing 50 mM K_2HPO_4/KH_2PO pH 6.5, 0.05% $Na_2S_2O_3$, 18 mM L-glutamate and 0.8 mM pyridoxal-5-phosphate. The reaction was stopped by placing the tubes in ice water and subsequent addition of 100 µl 0.4 M H_2SO_4. Afterwards the samples were centrifuged for 1 hr at 300 rpm. GABA was isolated from the supernatant and determined after reaction with *O*-phtalaldehyde according to Van der Heijden and Korf [8].

Data analysis

Comparison of the behavioural data was carried out using the Mann–Whitney U-test. Effects on temperature were tested statistically using the Student paired *t*-test. The effects on GABA-T and GAD were also tested on statistical significant differences using Student's *t*-test. A probability lower than 5% (two tailed) was considered to be significant.

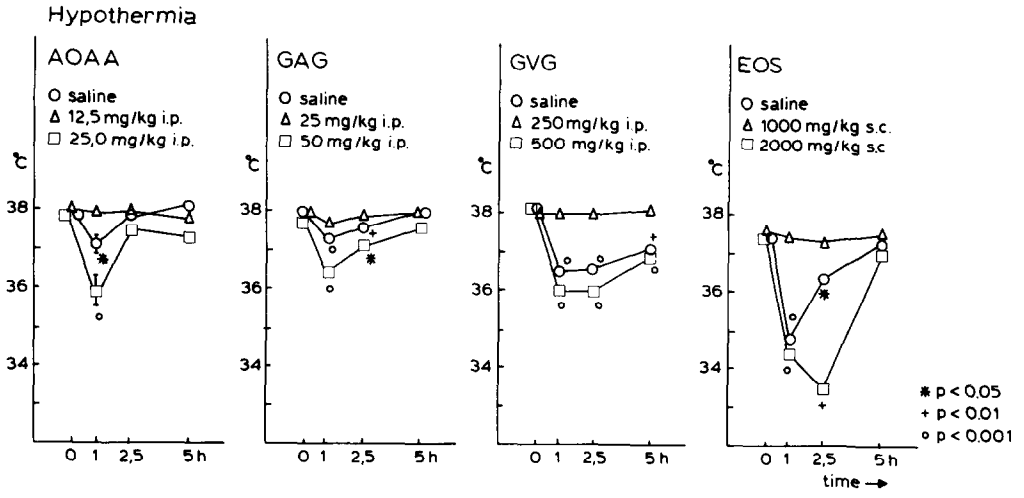

Fig. 1. The effect of GABA-T inhibitors on temperature. The compounds were administered intraperitoneally or subcutaneously and just before, and 1, 2.5 and 5 hr after, injection the rectal temperature was measured. Bars represent S.E.M. When no bar is present the S.E.M. is smaller than the symbol. Statistical significance was tested using the Student's paired *t*-test. Each group consisted of 6 animals.

RESULTS

Effects on temperature

All GABA-T inhibitors induced a hypothermia which was more pronounced after the highest dose used (Fig. 1). This effect was short lasting for EOS, AOAA and GAG. Using GVG a significant hypothermia was present after 1 hr and still found after 5 hr. EOS induced the strongest hypothermia with a decrease of 4° after 2.5 hr. No difference was found using this compound after 5 hr.

Effect on hole-board exploration

As can be seen in Fig. 2 all GABA-T inhibitors induced a decrease in exploration at one or both time-points studied. AOAA decreased exploration only after 5 hr in a dose of 25 mg/kg whereas the effects of GVG and EOS were only apparent after 2.5 hr. GAG decreased activity in the two doses used both after 2.5 and 5 hr. Only EOS influenced activity more than 50% in the highest dose used.

Effects on quasi-morphine withdrawal behaviour

The effects of the GABA-T inhibitors were different (see Fig. 3). AOAA (25 mg/kg) and GAG (25 and 50 mg/kg) decreased body shakes both after 2.5 and 5 hr whereas GVG and EOS had no effect. The highest doses used for AOAA, GAG and EOS also decreased locomotor activity after 2.5 hr, this effect, however, disappeared for GAG and EOS after 5 hr. On the contrary GVG in a dose of 250 mg/kg induced an increase in locomotor activity after 2.5 hr.

Effect on GABA-T and GAD activities

As can be seen in Fig. 4, GAD activity was decreased after treatment with GAG both in cortex and in cerebellum. In cortex a transient decrease was found after 2.5 hr using 25 mg/kg while after the higher dose (50 mg/kg) the effect after 5 hr was more pronounced. At the latter timepoint also an effect in cerebellum was found. GVG induced a

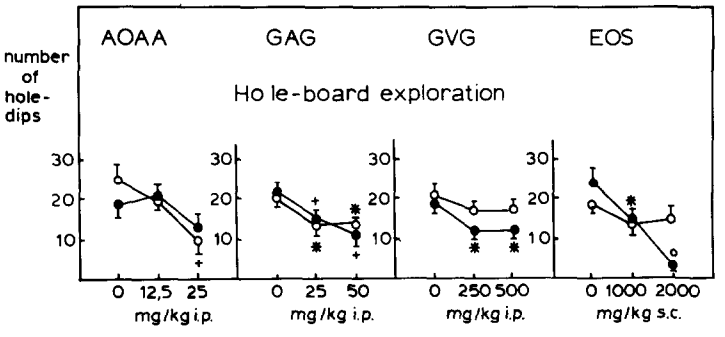

Fig. 2. The effect of GABA-T inhibitors on hole-board exploration. Rats were treated with saline or with one of the two doses of AOAA, GAG, GVG or EOS, and were placed after 2.5 or 5 hr on the hole-board. Hole dipping was scored automatically using photocells during 5 min. Bars represent S.E.M. (N = 6 animals per dose). Statistical significance was calculated using Mann–Whitney's *U*-test. * $P < 0.05$ (two-tailed); + $P < 0.02$; ○, $P < 0.005$; ●—●, 2.5 hr; ○—○, 5 hr.

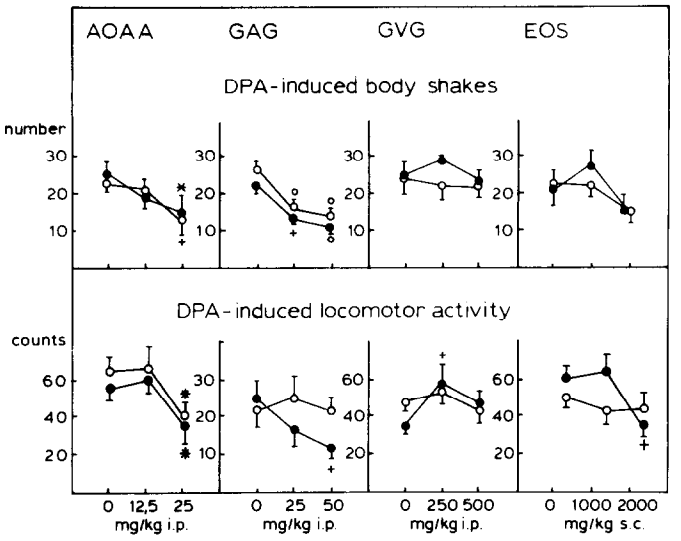

Fig. 3. The effect of GABA-T inhibitors on DPA-induced body shakes and DPA-induced locomotor activity. Rats were treated with saline or with one of the two doses of AOAA, GAG, GVG and EOS and received after 2.5 or 5 hr an injection of DPA (300 mg/kg i.p.) after which their behaviour was observed during 15 min. Bars represent S.E.M. (N = 6 animals per dose). Statistical significance was calculated using Mann–Whitney's *U*-test. * $P < 0.05$ (two-tailed); + $P < 0.02$; ○ $P < 0.005$; ●—● 2.5 hr; ○—○ 5 hr.

rather small decrease in cerebellum after 5 hr using both 250 and 500 mg/kg and only using 500 mg/kg in the cortex. EOS had no effect. Using AOAA 50 mg/kg a strong decrease was found after 2.5 hr in the cortex and a less decrease after 5 hr in this region, whereas in the cerebellum an increase was found after 5 hr.

The effects on GABA-T were most pronounced for GAG and AOAA (Fig. 5). The great variability in GABA-T activity (2-fold in the GAG experiment) is due to an unexplainable high extinction at 340 nm of the NADH reference solution resulting in a high molar activity. When expressed in arbitrary fluorimetric units such great differences were not present.

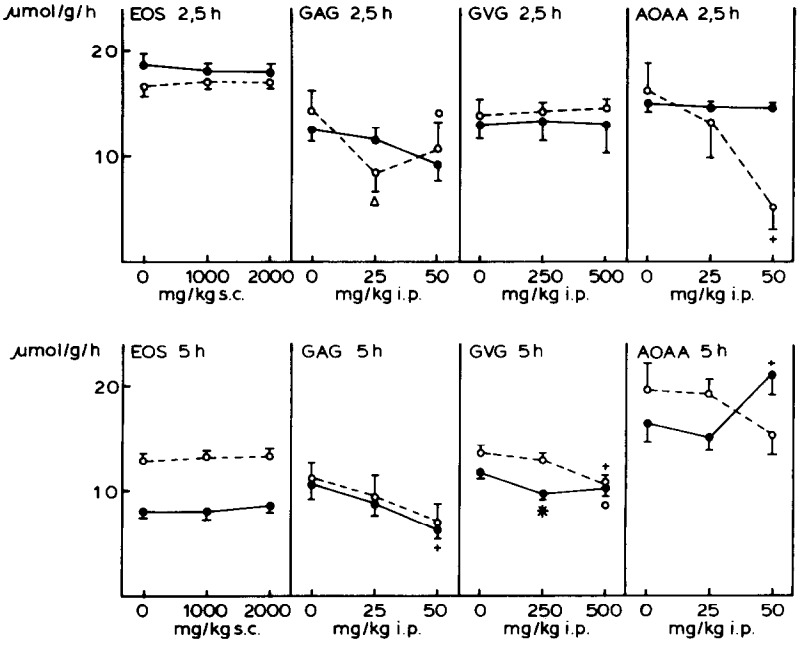

Fig. 4. The effect of GABA-T inhibitors of GAD-activity in cerebral cortex and cerebellum. Rats were treated with saline or with one of two doses of AOAA, GAG, GVG or EOS and were decapitated after 2.5 or 5 hr. GAD activity in cortex and cerebellum was measured as described under methods. Bars represent S.D. (N = 5 animals per group). Statistical significance was calculated using Student's *t*-test. ○ $P < 0.05$; + $P < 0.025$; △ $P < 0.001$; * $P < 0.005$; ●—●, cerebellum; ○---○, cortex.

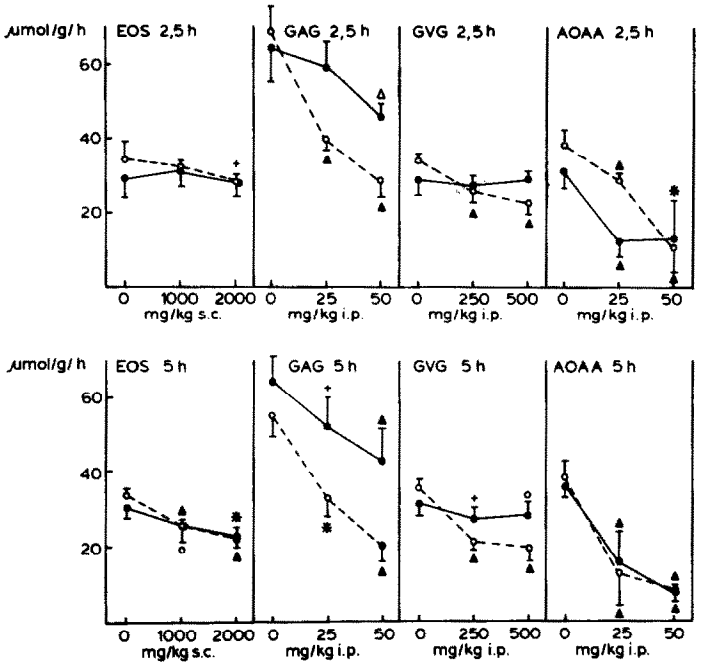

Fig. 5. The effect of GABA-T inhibitors on GABA-T activity in cerebral cortex and cerebellum. Rats were treated with saline or with one of two doses of AOAA, GAG, GVG or EOS and were decapitated after 2.5 or 5 hr. GABA-T activity was measured in cortex O---O and cerebellum ●—● as described under methods. Bars represent S.D. (N = 5 animals per group). Statistical significance was calculated using Students's *t*-test; O $P < 0.05$; + $P < 0.025$; △ $P < 0.01$; * $P < 0.005$; ▲ $P < 0.001$.

(The assays for all groups were done in one week.)

GAG and GVG exerted a stronger effect on cortex tissue, whereas EOS and AOAA were equally effective in both areas. The inhibitory effects were in all cases higher after 5 hr.

DISCUSSION

The present data confirm the sedative and hypothermic actions of GABA-T inhibitors in animals [9–13]. For AOAA and GAG no parallel between hypothermia and the decrease in hole-board exploration is present. Rectal temperature was returned to normal after 5 hr, when sedation was still present. It has been shown that the hypothermic action of GAG, GVG and AOAA can be reversed by increasing the ambient temperature [9, 13]. Data about effects on locomotor activity were, however, not available. For the other compounds EOS and GVG a decrease in exploration was only found after 2.5 hr when a strong hypothermia was present. It is, therefore, possible that the sedation found after administration of the latter compounds was secondary to their effects on temperature.

Neither the sedative nor the hypothermic effects correlate with the effects on DPA-induced quasimorphine withdrawal behaviour, measured as body shakes and enhanced locomotor activity. Administration of GAG induced a decrease in body shakes after both 2.5 and 5 hr, not in locomotor activity after 5 hr. Thus, the decrease in exploration in one experiment coincides with the decrease in DPA-induced body shakes but not with the decrease in DPA-induced locomotion observed in another experiment. Lowering of the temperature and the low level of exploration induced by GVG after 2.5 hr was in sharp contrast with an increase in DPA locomotor activity. In view of the low activity of the control group in this experiment this increase may be a rather accidental finding. The score of control groups in the AOAA and EOS experiments are in the same order of magnitude as the low dose GVG group. The strong effects of EOS on temperature and exploration were only parallel to a small decrease in locomotion after 2.5 hr. No effect on body shakes was found despite the fact that body shakes may be related to temperature phenomena [14].

The effects of AOAA on DPA induced behaviour were less pronounced than reported by De Boer *et al.* [2]. No explanation can be given for this difference.

The absence of a correlation between the effects of the GABA-T inhibitors on exploration and on DPA-induced locomotor activity is in contrast with data about α_2-adrenergic agonists [15]. Using these compounds a high correlation was found between the ED_{50}s for the decrease in DPA-induced locomotor activity and in exploration on a hole-board.

The potentiation of body shakes induced by DPA, observed after treatment of rats with EOS [16] was not seen in our study. The most obvious difference with this report is the chronic character of treatment. Also using GVG an increase in DPA induced behaviour can be observed when a longer pre-treatment time has been used [17].

The inhibitory effects on GABA-T activity of the compounds were less comparable than expected.

The doses used were chosen to inhibit GABA-T activity in rat brain for 60–80% [18–20]. The doses for GVG and EOS were derived from comparative data on mouse brain [6, 21, 22].

However, GVG and EOS were obviously less effective than GAG and AOAA despite the much higher doses. Small regional differences appear to be present for GAG and GVG but not for EOS and AOAA. It is possible that differences in distribution can be held responsible for this fact. Differences in regional increases in GABA concentration were reported for AOAA and GVG [19].

GAG and GVG were found to inhibit GAD also, the first compound being more effective, confirming data in mice [21, 22]. The increase in GAD 5 hr after AOAA found in the cerebellum may be an artefact. AOAA has been reported to inhibit GAD, when added *in vitro*. However, after administration *in vivo*, several authors could not find an inhibition [20, 23], although others have described such an effect using rather low doses [24, 25] in agreement with our data in cortex 2.5 hr after AOAA treatment.

The inhibition of GABA-T after 5 hr seems to be related to the sedative effect on this time. No relation could be found between the biochemical effects and hypothermia. It can be suggested that effects in other, non-analysed, parts of the brain, e.g. hypothalamus, are more important.

As the DPA-induced body shakes have been thought to be caused by an enhanced GABAergic transmission [1, 2], the suppressive effect of AOAA on these symptoms has given rise to the hypothesis that DPA and AOAA should act in different compartments (see the Introduction). We have tested whether other GABA-T inhibitors would induce also such a decrease in DPA-induced body shakes. The data concerning the inhibition of GABA-T indicate that the compounds having a low effect on GABA-T activity had a low effect on behaviour also, which finding is not in contrast with the hypothesis stated above. It is possible that higher doses of GVG and EOS leading to a stronger inhibition of GABA-T may have a stronger effect on DPA-induced body shakes. However, doubling of the dose of GVG (1000 mg/kg) did not decrease DPA-induced body shakes when administered 12 hr before DPA [17]. Since inhibition of GABA-T will be maximal at this time [22], this factor cannot be held responsible for this decrease. Therefore, the data may be explained alternatively as an effect of GAD-inhibition. Only inhibitory effects on body shakes were found for AOAA and GAG which compounds strongly affect GAD in the cortex. This explanation suggests a direct counteraction in the nerve terminal compartment of the GABA-increasing action of DPA. Direct GAD-inhibition by 3-mercaptopropionic acid decreased DPA-induced body shakes also [2]. These data support the hypothesis that DPA exerts its action to induce quasi-morphine withdrawal behaviour by increasing the GABA-concentration in nerve terminals [2, 26].

Since GABA-T inhibitors will initially induce an increase in GABA in other compartments, one can explain why these compounds do not induce quasi-morphine withdrawal behaviour.

In conclusion, the effects of the four GABA-T inhibitors on DPA-induced quasi-morphine withdrawal behaviour, appear to be hardly related to sedative or hypothermic effect nor caused by GABA-T inhibition itself. It is possible that concurrent GAD inhibition rather than GABA-T inhibition must be held responsible for these effects.

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