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# Effects of acute and subacute treatment with ergot drugs on the GABA system in specific brain regions

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The effect of dihydroergotoxine and dihydroergotamine on γ-aminobutyric acid (GABA) levels, the aminooxyacetic acid (AOAA)-induced accumulation of GABA, and the in vitro activity of L-glutamate decarboxylase (GAD) have been examined in various regions of rat brain. Dihydroergotoxine (1 mg $kg^{-1}$) decreased the concentration of GABA and enhanced the AOAA-induced accumulation of GABA in the caudate nucleus and cingulate cortex. Dihydroergotoxine 10·0 mg $kg^{-1}$ decreased the AOAA-induced accumulation of GABA in the substantia nigra. The repeated treatment with dihydroergotoxine, 0·05 mg $kg^{-1}$ for eight days, also decreased the concentration of GABA in the cingulate cortex and diminished the AOAA-induced accumulation of GABA in the substantia nigra. The administration of 0·1 mg $kg^{-1}$, but not higher doses; of dihydroergotamine, enhanced the AOAA-induced accumulation of GABA in the cingulate cortex. Dihydroergotamine (10·0 mg $kg^{-1}$) decreased the concentration of GABA in the cingulate cortex and increased the AOAA-induced accumulation of GABA in the caudate nucleus. The activity of GAD in the cingulate cortex, but not in the caudate nucleus, was enhanced after a high dose of dihydroergotamine. Observed increases in the AOAA-induced accumulation of GABA indicate that dihydroergotoxine and dihydroergotamine in at least some brain areas cause an apparent increase in GABA synsynthesis in vivo, which is presumably a compensatory phenomenon due to a diminished GABAergic transmission under the influence of these drugs.

Ergot alkaloids known to interact with central and peripheral neurotransmission, are being used as therapeutic agents in various disorders (Hoffbrand et al 1976) but their actions on the central nervous system are not fully understood. These drugs possess agonist and antagonist activities with respect to dopaminergic and α-adrenergic receptors (Greenberg et al 1976; Lew et al 1976; Goldstein et al 1978). Dihydrogenated ergot alkaloids stimulate central 5-HT receptors (Loew et al 1976). To further elucidate the action of ergot drugs on the central nervous system we have studied the effects of two ergot alkaloids—dihydroergotoxine and dihydroergotamine on the GABA system in specific brain regions. Because of the multiple neuronal interactions in the brain and especially of a dopaminergic-GABA ergic interaction in the extrapyramidal (Precht & Yoshida 1971; Kim & Hassler 1975; Marco et al 1978; Fuxe et al 1979; Perićić & Walters 1980) and limbic system (Fuxe et al 1975), it was reasonable to expect that the ergot drugs mentioned would affect if not directly, indirectly the activity of the GABA system. The effects of dihydroergotoxine and dihydroergotamine were studied in the caudate nucleus and cingulate cortex. The caudate nucleus contains cell bodies of GABA neurons projecting to the substantia nigra and globus pallidus and also some short axoned GABA interneurons. In the cingulate cortex, which belongs to the limbic system and presumably contains GABA interneurons, the effects of many drugs on the GABA system have been more pronounced than in other brain regions (Perićić et al 1977, 1978). Further, the effects of dihydroergotoxine were studied in the substantia nigra and in the cerebellar cortex, brain regions containing GABA terminals and several types of GABA neurons, respectively. Since the measurement of neurotransmitter turnover gives a better insight into the activity of the corresponding neurons than the determination of neurotransmitter levels, the effects of dihydroergotoxine and dihydroergotamine, not only on neurotransmitter concentration but also on the amino-oxyacetic acid (AOAA)-induced accumulation of GABA and on the activity of glutamate decarboxylase (GAD), were studied. AOAA preferentially inhibits GABA catabolism and induces a linear increase of GABA (Walters et al 1978).

## MATERIALS AND METHODS

Male Wistar rats, 160–200 g were used. Aminooxyacetic acid hemihydrochloride (AOAA, Eastman Kodak Co., Rochester, NY, USA) was dissolved in

0·9% NaCl (saline) and adjusted to neutral pH before administration. Dihydroergotoxine methane sulphonate and dihydroergotamine methane sulphonate (Lek, Ljubljana, Yugoslavia) were administered intraperitoneally (i.p.) 60 min and AOAA also i.p. 70 min before death.

For determination of GABA levels, animals were killed by exposing their heads to a focussed beam of microwave irradiation for 5·0 s (Guidotti et al 1974). The brains were placed, ventral side up, in a tissue slicer with a 22 mm long brain shape depression and slits for razor blades at 1·5 mm intervals. Bilateral punches of caudate nucleus, cingulate cortex, and, in some experiments, of substantia nigra and cerebellar cortex were obtained from 3 mm thick transversal slices as described elsewhere (Peričić et al 1977). Approximately 5 mg of brain tissue was homogenized immediately in 300 $\mu$l of 0·1 M HCl. GABA was determined by a modification (Peričić et al 1977) of the enzymatic fluorimetric method of Hirsh & Robins (1962).

For estimation of L-glutamate decarboxylase (GAD) activity as described by Miller et al (1978), tissue was punched from the cingulate cortex and caudate nucleus and homogenized in 250 $\mu$l of 0·05 M-imidazole acetate buffer, pH 6·5, containing 10 mM-mercaptoethanol and 0·5% Triton X-100. GAD activity was measured during a 5 min incubation period in a total incubation volume of 50 $\mu$l using a subsaturating 5 mM concentration of L-[U-$^{14}$C]glutamic acid. The [$^{14}$C]GABA formed was isolated by Dowex column chromatography, eluted into 4 ml aquasol and counted in a liquid scintillation counter. Protein concentrations were determined in 10 $\mu$l of the homogenates according to Lowry et al (1951). Data were analysed using a two tailed Student's *t*-test with a criterion for significance of $P < 0.05$.

## RESULTS

Treatment of animals with 25 mg kg$^{-1}$ AOAA resulted in a significant increase of GABA concentration in the cingulate cortex and caudate nucleus (Fig. 1). When 1 mg kg$^{-1}$ dihydroergotoxine was given in combination with AOAA, the AOAA-induced accumulation of GABA in these brain regions was significantly increased. As shown in Fig. 1, treatment of animals with dihydroergotoxine alone (1·0 mg kg$^{-1}$) had an opposite effect; it decreased the concentration of GABA in the cingulate cortex and caudate nucleus. A dose of dihydroergotoxine that increased the AOAA-induced accumulation of GABA in the regions mentioned did not have a similar effect in the substantia nigra and cerebellar cortex (Fig. 2) whereas 10·0 mg kg$^{-1}$ of dihydroergotoxine significantly depressed this accumulation in the substantia nigra but still did not show any change in the cerebellar cortex (Fig. 2).

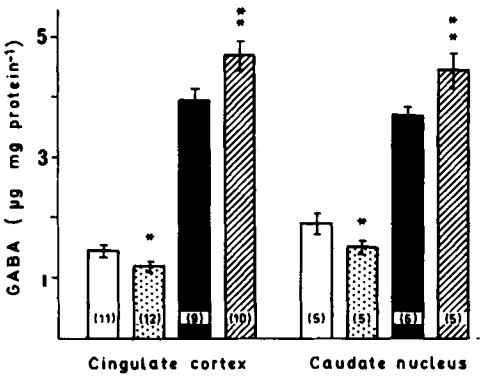


FIG. 1. Effect of dihydroergotoxine on GABA levels and on the aminooxyacetic acid (AOAA)-induced accumulation of GABA in the cingulate cortex and caudate nucleus. Dihydroergotoxine (1·0 mg kg$^{-1}$ i.p.) was administered 60 min and AOAA (25 mg kg$^{-1}$ i.p.) 70 min before death. Bars represent mean ± s.e.m. Numbers in the bars represent number of experiments. Columns: open, control; dotted, dihydroergotoxine; shaded, AOAA; hatched, AOAA + dihydroergotoxine. *significantly different from control ($P < 0.05$ or $P < 0.02$) ** significantly different from AOAA treated group ($P < 0.05$).

When animals were treated repeatedly, over eight days with 0·05 mg kg$^{-1}$ of dihydroergotoxine, the concentration of GABA in the cingulate cortex, but

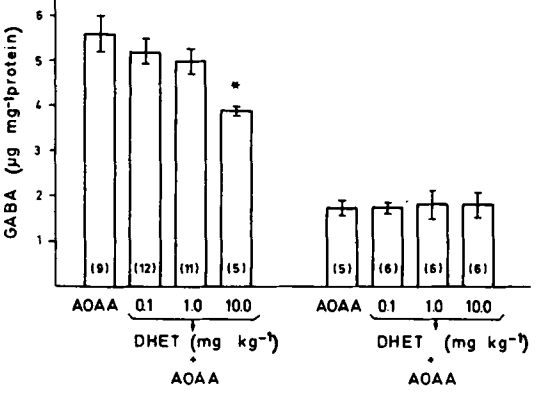


FIG. 2. Effect of dihydroergotoxine on the aminooxyacetic acid (AOAA)-induced accumulation of GABA in the substantia nigra and cerebellar cortex. Different doses of dihydroergotoxine were administered i.p. 60 min and AOAA (25 mg kg$^{-1}$ i.p.) 70 min before death. Bars represent mean ± s.e.m. Numbers in the bars represent number of experiments. * $P < 0.01$ when compared with AOAA alone.

not in the caudate nucleus or substantia nigra, was still significantly depressed. Unlike the acute treatment, the repeated treatment with dihydroergotoxine did not change the AOAA-induced accumulation of GABA in the cingulate cortex and caudate nucleus but depressed this accumulation in the substantia nigra. (Fig. 3).

The effects of dihydroergotamine (0·1, 1·0 and 10·0 mg $kg^{-1}$) administration on the concentration of GABA and the AOAA-induced accumulation of GABA in the caudate nucleus and cingulate cortex are shown in Table 1. Neither of these doses produced significant changes in GABA levels in the caudate nucleus, but the 10·0 mg $kg^{-1}$ dose decreased the levels of GABA in the cingulate cortex. At 0·1 mg $kg^{-1}$ dihydroergotamine increased the AOAA-induced accumulation of GABA in the cingulate cortex, but had no effect in the caudate nucleus. A dose of 1·0 mg $kg^{-1}$ did not change the AOAA-induced accumulation of GABA in the brain regions studied, while 10·0 mg $kg^{-1}$ increased this accumulation in the caudate nucleus, but not in the cingulate cortex.

The activity of GAD in the caudate nucleus and cingulate cortex was not changed after treatment with dihydroergotoxine, but dihydroergotamine significantly increased GAD activity in the cingulate cortex (Table 2).

Table 1. Effect of dihydroergotamine on GABA levels and on the amino-oxyacetic acid (AOAA)-induced accumulation of GABA in two rat brain regions

| Treatment | GABA (μg $mg^{-1}$ protein) Caudate nucleus | | Cingulate cortex | |
|---|---|---|---|---|
| Control | 1·79 ± 0·07 | (11) | 1·56 ± 0·07 | (11) |
| Dihydroergotamine (0·1 mg $kg^{-1}$) | 1·64 ± 0·04 | (5) | 1·48 ± 0·01 | (6) |
| Dihydroergotamine (1·0 mg $kg^{-1}$) | 1·60 ± 0·06 | (6) | 1·53 ± 0·09 | (11) |
| Dihydroergotamine (10·0 mg $kg^{-1}$) | 1·59 ± 0·10 | (6) | 1·27 ± 0·05* | (10) |
| AOAA | 2·98 ± 0·06† | (10) | 3·82 ± 0·17† | (10) |
| AOAA + dihydroergotamine (0·1 mg $kg^{-1}$) | 2·95 ± 0·29 | (6) | 5·98 ± 0·10*** | (6) |
| AOAA + dihydroergotamine (1·0 mg $kg^{-1}$) | 3·10 ± 0·27 | (6) | 3·88 ± 0·23 | (6) |
| AOAA + dihydroergotamine (10·0 mg $kg^{-1}$) | 3·47 ± 0·14** | (11) | 3·97 ± 0·10 | (10) |

Different doses of dihydroergotamine were administered i.p. 60 min, and AOAA (25 mg $kg^{-1}$ i.p.) 70 min before death. The results are the mean ± s.e.m. of (n) experiments. *$P$ <0·01 when compared with control group; **$P$ < 0·01 when compared with AOAA treated group; ***$P$ < 0·001 when compared with AOAA treated group; †$P$ <0·001 when compared with control group.

## DISCUSSION

The results suggest that dihydroergotoxine and dihydroergotamine affect GABAergic transmission in the brain. A decrease of GABA concentration was obtained in the cingulate cortex and caudate nucleus after 1·0 mg $kg^{-1}$ dihydroergotoxine. The same dose increased the AOAA-induced accumulation of GABA in these brain regions while 10·0 mg $kg^{-1}$ was necessary to obtain a significant change of the AOAA-induced accumulation of GABA in the substantia nigra. However, compared with the other increases mentioned, this change was in an opposite direction. The accumulation of GABA in the cerebellar cortex (164% of control) was not affected by dihydroergotoxine. Although GABA neurons in the cerebellar cortex play an important physiological

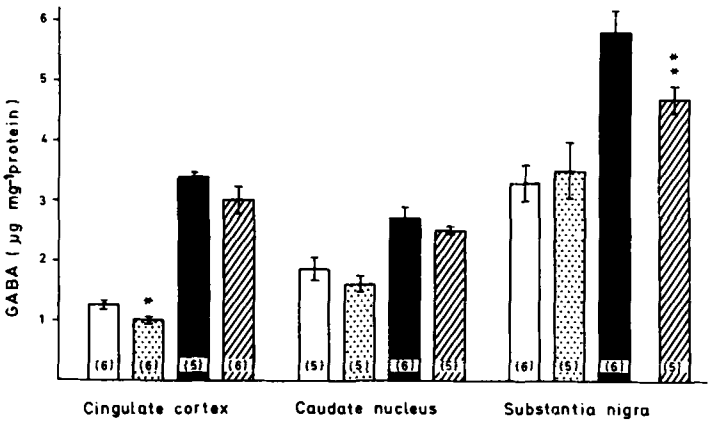


FIG. 3. Effect of repeated doses of dihydroergotoxine on GABA levels and on the aminooxyacetic acid (AOAA)-induced accumulation of GABA in specific brain regions. Dihydroergotoxine (0·05 mg $kg^{-1}$ i.p.) was administered over a period of eight days. Sixty min after the last injection the animals were killed. AOAA (25 mg $kg^{-1}$ i.p.) was administered 70 min before death. Bars represent mean ± s.e.m. Numbers in the bars represent number of experiments. Columns: open, control; dotted, dihydroergotoxine, 0·05 mg $kg^{-1}$ for eight days; shaded, AOAA; hatched, dihydroergotoxine (0·05 mg $kg^{-1}$ for eight days) + AOAA. * significantly different from control ($P$ <0·02); ** significantly different from AOAA treated group ($P$ <0·05).

role (Ito 1976), and the cerebellum contains the highest density of GABA receptor sites (Zukin et al 1974), the effects of many drugs on the GABA system in this brain region are not seen or are less pronounced than in other brain regions (Perićić et al 1977, 1978). Even though the technique of AOAA administration has definite limitations, this technique might provide a simple means of screening for in vivo drug induced alterations in GABA synthesis (Perićić et al 1978). Apparent changes in GABA synthesis caused by drugs that affect GABA-mediated neurotransmission have been demonstrated with this technique (Perez de la Mora et al 1975; Fuxe et al 1975; Hökfelt et al 1976; Perićić et al 1977, 1978). Accordingly, the observed changes in the AOAA-induced accumulation of GABA suggest that dihydroergotoxine increases the synthesis of GABA in the cingulate cortex and caudate nucleus, and decreases it in the substantia nigra. An enhancement of GABA synthesis has previously been observed after GABA receptor blocking drugs (Wood & Peesker 1975; Perićić et al 1978), whereas an opposite effect has been obtained after drugs that either enhance or mimic the function of GABA (Perićić et al 1977; Moroni et al 1979). The fact that GABA systems in the substantia nigra and cerebellar cortex react differently to the drug treatment is not new, and has been observed after drugs such as benzodiazepines, neuroleptics, morphine etc. (Perićić et al 1977; Mao & Costa 1978; Perićić & Walters 1980; Moroni et al 1978). The differences obtained between the brain areas examined are presumably due to the different neuronal loops and/or different mechanisms regulating the function of GABA-containing elements in them. Thus, it is assumed that GABA metabolism in different brain regions may be regulated by dopaminergic, cholinergic, enkephalinergic and perhaps glutamatergic neurons (Moroni et al 1978).

The apparent diminution of GABA synthesis in the substantia nigra was also observed after eight days of repeated treatment with very low doses of dihydroergotoxine (0·05 mg $kg^{-1}$), whereas increases in the AOAA-induced accumulation of GABA observed in the caudate nucleus and cingulate cortex after the acute treatment disappeared after the prolonged treatment (eight days). The significance of the reduced synthesis of GABA elicited by acute and subacute treatment with dihydroergotoxine in the substantia nigra is difficult to assess. The observed decrease of GABA concentration in the cingulate cortex also remained after eight days treatment with dihydroergotoxine, whereas the decrease of GABA in the caudate nucleus did not reach the level of significance after the prolonged treatment.

Table 2. Effect of dihydroergotoxine and dihydroergotamine treatment on GAD activity in the caudate nucleus and cingulate cortex

| Treatment | GAD activity (nmol GABA $mg^{-1}$ protein/5 min) Caudate nucleus | Cingulate cortex |
|---|---|---|
| Control | 9·26 ± 0·48 | 4·95 ± 0·24 |
| Dihydroergotoxine | 9·72 ± 0·30 | 5·55 ± 0·15 |
| Dihydroergotamine | 9·56 ± 0·36 | 5·74 ± 0·15* |

Dihydroergotoxine (50 mg $kg^{-1}$) and dihydroergotamine (50 mg $kg^{-1}$) were administered i.p. 60 min before death. The results are the mean ± s.e.m. of 6 experiments. * $P < 0·05$ when compared with control.

Differences between brain areas in the reaction of their GABA systems to the drug treatment were also present after dihydroergotamine administration. Whereas 1·0 and 10·0 mg $kg^{-1}$ failed to change the concentration of GABA in the caudate nucleus, 10·0 mg $kg^{-1}$ decreased the GABA level in the cingulate cortex. Moreover, in the cingulate cortex an elevation of the AOAA-induced accumulation of GABA was observed only after a very low dose of dihydroergotamine (0·1 mg $kg^{-1}$). With this dose the analogous change was not observed in the caudate nucleus. On the contrary, 10·0 mg $kg^{-1}$ of dihydroergotamine enhanced the AOAA-induced accumulation of GABA only in the caudate nucleus. Accordingly, it appears that only certain doses of dihydroergotamine accelerate the synthesis of GABA in the regions mentioned. Although we do not have an adequate explanation for this, it is worth mentioning that dihydroergotamine had a biphasic effect on the dopaminergic system. Low doses of the drug enhanced, while high doses decreased the synthesis of dopa in the striatum (Živković, personal communication). An activation of GAD was also found in the cingulate cortex after dihydroergotamine. The fact that the similar elevation of GAD activity was not obtained in vitro, i.e. in the homogenates obtained from the caudate nucleus and cingulate cortex after dihydroergotoxine administration, is not surprising and still does not rule out the possibility that drug-induced changes in the saturation of GAD by cofactor may be occurring in vivo. It has been suggested that GABA synthesis and GAD activity may be regulated by factors altering the binding of the PLP cofactor to GAD (Miller et al 1977).

Much higher doses of ergot drugs are required to produce significant changes in the activity of neuro-

transmitter systems other than GABA (Loew et al 1976; Bürki et al 1978; Vigouret et al 1978) so the results obtained on the GABA system seem to be meaningful for the understanding of the central action of these ergot drugs. Since both drugs are often used therapeutically it would be useful to know whether these results could have practical implications. Although this might be difficult to assess, both drugs examined shorten the latency of allylglycine-induced convulsions, dihydroergotoxine being the more potent. It has also accelerated the onset of picrotoxin-induced convulsions and when administered in very low doses it lowered the ED50 for picrotoxin (Peričić 1980). Whether these effects are the result of the observed decreases of GABA levels and of the apparent changes in GABA synthesis observed in some brain regions after administration of dihydroergotoxine, is not yet clear. From these results and the fact that an enhancement of GABA synthesis has previously been observed after administration of drugs that block GABAergic transmission (Wood & Peesker 1975; Peričić et al 1978), the impression cannot be avoided that this drug decreases GABAergic function and therefore by a compensatory mechanism, increases the synthesis of GABA. The lower concentrations of GABA observed in the caudate nucleus and cingulate cortex after dihydroergotoxine, simultaneously with the apparent increases in the synthesis of GABA, might indicate that the release of GABA is enhanced even more than GABA synthesis. Hence it can be speculated that dihydroergotoxine diminishes GABAergic function by some other mechanism like altering the uptake of GABA or by interfering with the interaction of GABA with its receptor. Anyway, the sedative effects produced by these drugs do not seem to be associated with an enhancement of GABAergic transmission. In spite of the regional differences observed in the effects of dihydroergotoxine and dihydroergotamine on GABA level and GABA synthesis, the general impression is that both of these drugs diminish GABAergic transmission in at least some brain regions.

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