GABA-ERGIC EFFECTS OF HARMANE INDEPENDENT OF ITS ACTION ON BENZODIAZEPINE RECEPTORS

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Among the many "endogenous ligands of benzodiazepine receptors" β-carbolines [7] and, in particular, harmane [11] and norharmane [5], exhibit the highest activity as substances competitively inhibiting specific binding of labeled benzodiazepine tranquilizers (BDT) by brain membranes. In this way the pharmacologic effects of harmanes can be regarded as the result of their primary action on benzodiazepine receptors [12]. The effects of harmane are very varied: in large doses it induces tremor seizures [2, 13], which are abolished by diazepam [12], in subtoxic doses it counteracts the most important effects of BDT [15], but at the same time inhibits locomotion and orienting behavior of animals and potentiates the narcotic effect of barbiturates, but in small doses (1 mg/kg) it possesses intrinsic anxiolytic activity [2].

The mechanism of BDT-like effects of harmane can be regarded by analogy with the action of BDT. The latter potentiate GABA-ergic inhibition [3, 10] by increasing the affinity of GABA receptors for GABA [1, 8] as a result of the primary action of BDT on "benzodiazepine receptors" coupled with GABA receptors [9]. Within a narrow range of concentrations (10^{-7} — 10^{-5} M) harmane also exhibits a GABA-potentiating effect on brain neurons [4].

The present investigation showed that harmane can not only have a direct GABA-potentiating action, but it can also enhance GABA-ergic inhibition in the CNS, increasing mediator release by GABA-ergic neurons irrespective of its effect on benzodiazepine receptors.

EXPERIMENTAL METHOD

The effect of GABA, chlordiazepoxide (elenium, from Poland), harmane, and some of its deriviatives was studied on release of radioactive label from slices of cerebral cortex, preincubated with [^{3}H]-GABA, both spontaneous (basal) and induced by electrical stimulation. The method described previously [14] was used. Thin slices (200-250 μ) of rat cerebral cortex were incubated for 30 min with [^{3}H]-GABA (specific activity 0.25 mCI, New England Nuclear) in a concentration of 10^{-7} M in the presence of depakine (10^{-5} M; an inhibitor of GABA transaminase) and β-alanine (10^{-3} M; an inhibitor of glial uptake of GABA), washed three times with cold incubation medium (without [^{3}H]-GABA, 5 ml), and transferred to polyethylene grids of 0.5-ml perfusion chambers, and placed between two platinum electrodes. The slices were perfused with solution of the following composition (in mM): NaCl — 122, KCl — 3.1, $CaCl_2$ — 1.3, $MgSO_4$ — 1.2, KH_2PO_4 — 0.4, $NaHCO_3$ — 2.5, glucose 10, ascorbic acid 1.14, depakine 0.01, β-alanine 1; pH 7.4. The rate of perfusion was 0.5 ml/min. Five-minute portions of perfusate were collected starting from 30 min after the beginning of perfusion, i.e, from the time when spontaneous elution of the radioactive label flattened out on a plateau. At the 45th and 60th minutes the slices were stimulated by square pulses (5 Hz, 2 msec, 12 mA) for 2 min (S_1 and S_2). The substances for testing were added to the medium 13 min before S_2; the duration of perfusion with the substances was 15 min. Radioactivity of the samples was measured with an SBS-2 scintillation counter. The outflow of radioactive label during electrical stimulation was expressed as a coefficient of release. The results were subjected to statistical analysis by the usual methods.

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TABLE 1. Effect of Test Substances on Release of [^{3}H]-GABA by Slices of Rat Cerebral Cortex, Spontaneous and Induced by Electrical Stimulation

Substance and concentration (in M)	Number of measurements	Release in response to stimulation S_2S_1	Basal release ($\bar{x} - S_{\bar{x}} \cdot t$)			
			45/40 min	50/45 min	55/50 min	60/55 min
Control	24	0,98±0,03	0,97±0,04	0,99±0,03	1,00±0,03	0,99±0,04
GABA, $3 \cdot 10^{-5}$	8	0,42±0,05	—	—	—	—
Picrotoxin, 10^{-6}	14	1,38±0,05	1,18±0,09	1,18±0,05	1,16±0,05	1,18±0,09
GABA, $3 \cdot 10^{-5}$ + picrotoxin, 10^{-6}	8	0,80±0,05	—	—	—	—
Chlordiazepoxide, 10^{-6}	8	1,06±0,08	1,01±0,06	0,98±0,04	1,07±0,03	1,02±0,08
Chlordiazepoxide, $3 \cdot 10^{-5}$	10	1,02±0,06	—	—	—	—
GABA, $3 \cdot 10^{-5}$ + chlordiazepoxide, 10^{-6}	8	0,48±0,03	—	—	—	—
GABA, $3 \cdot 10^{-5}$ + chlordiazepoxide, $3 \cdot 10^{-5}$	6	0,40±0,05	—	—	—	—
Harmane, 10^{-6}	8	1,18±0,06	—	—	—	—
Harmane, $3 \cdot 10^{-5}$	8	1,65±0,08	1,06±0,05	1,10±0,03	1,12±0,08	1,10±0,06
GABA, $3 \cdot 10^{-5}$ + harmane, 10^{-6}	8	0,84±0,03	—	—	—	—
GABA, $3 \cdot 10^{-5}$ + harmane, $3 \cdot 10^{-5}$	6	1,28±0,05	—	—	—	—
3-Methylharmane, $3 \cdot 10^{-5}$	8	1,70±0,03	—	—	—	—
GABA, $3 \cdot 10^{-5}$ + 3-methylharmane, $3 \cdot 10^{-5}$	8	1,22±0,08	—	—	—	—
C-383, $3 \cdot 10^{-5}$	12	1,96±0,06	1,12±0,08	1,18±0,1	1,16±0,1	1,14±0,08
GABA, $3 \cdot 10^{-5}$ + C-383, $3 \cdot 10^{-5}$	8	1,44±0,04	—	—	—	—
C-394, $3 \cdot 10^{-5}$	6	1,02±0,1	—	—	—	—
GABA, $3 \cdot 10^{-5}$ + C-394, $3 \cdot 10^{-5}$	8	0,44±0,06	—	—	—	—

EXPERIMENTAL RESULTS

GABA (3×10^{-5} M) inhibited release of radioactive label, induced by electrical stimulation, from slices of cerebral cortex preincubated with [^{3}H]-GABA by 58%. The inhibitory effect of GABA on pulsed release of [^{3}H]-GABA was abolished by picrotoxin (10^{-6} M) which, in the same concentration, itself increased the release of label induced by electrical stimulation from the slices (by 38%) and increased the basal release a little also (Table 1).

Chlordiazepoxide (10^{-6} and 3×10^{-5} M) had no significant effect on spontaneous and pulsed release of [^{3}H]-GABA, nor did it change the self-inhibiting effect of GABA (Table 1). This result agrees with data in the literature [6] and is evidence of the absence of BDT-binding sites (benzodiazepine receptors) in macromolecules of GABA autoreceptors.

Unlike chlordiazepoxide, harmane in concentrations of 10^{-6} and 3×10^{-5} M potentiated release of [^{3}H]-GABA induced by electrical stimulation by slices of cortex by 18 and 65% respectively, but increased spontaneous release of the label by only 6-12%. In the same concentrations harmane completely abolished the inhibitory effect of GABA on release of [^{3}H]-GABA induced by electrical stimulation by brain slices. 3-Methylharmane had a similar action (Table 1). Even greater activity was exhibited by another harmane derivative, C-383 which, in a concentration of 3×10^{-5} M, not only abolished the inhibitory effect of GABA on the pulsed release of [^{3}H]-GABA but alone it amost doubled (increased by 94%) release of [^{3}H]-GABA induced by electrical stimulation, although its spontaneous release was increased by only 12-18% (Table 1). However, not all harmane derivatives had a similar effect on the pulsed release of mediator by GABA-ergic neurons: C-394 did not change the release of radioactive label induced by electrical stimulation from brain slices preincubated with [^{3}H]-GABA, nor did it abolish the self-inhibiting effect of GABA.

Harmane and some of its derivatives thus specifically potentiate release of [^{3}H]-GABA by terminals of GABA-ergic neurons in the rat cerebral cortex induced by electrical stimulation and they abolish the inhibitory effect of GABA on pulsed release of [^{3}H]-GABA, despite the fact that GABA autoreceptors controlling pulsed release of the mediator are not coupled with benzodiazepine receptors, as is the case in postsynaptic GABA receptors (GABA–BDT receptor complexes). Irrespective of its effect on benzodiazepine receptors releasing GABA the action of harmane may be due to its ability to block potassium channels [4] in membranes of axons of GABA-ergic neurons, and so to increase frequency of spikes generated by these neurons in response to electrical stimulation.

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ACTION OF ETHMOZINE AND ETHACIZINE ON DOPAMINERGIC ADENYLATE CYCLASE OF THE BRAIN STRIATAL SYSTEM

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Ethmozine and ethacizine (the diethylamino analog of ethmozine) are the first phenothiazine derivatives to be introduced into medical practice for the treatment of arrhythmias [2-4, 6]. These substances have a marked cardiotropic action — their antiarrhythmic effects develop as a result of their direct effect on the heart [3, 4].

Many compounds of the phenothiazine group are known to be neuroleptics [5]. During clinical trials of ethmozine and ethacizine no marked neuroleptic or other psychotropic effect was found. This fact can be explained on the grounds that either these compounds do not pass through the blood—brain barrier or, unlike chlorpromizine, trifluoroperazine, and other phenothiazines, they have low affinity for receptors of the mediator systems of the brain.

One target for the action of neuroleptics of the phenothiazine series is the dopamine-sensitive adenylate cyclase system of the brain, coupled with the dopamine D-1 receptor [7, 9, 10-12]. Although the role of the dopaminergic adenylate cyclase system and of the dopamine D-1 receptor in the brain is not sufficiently clear, there is much evidence to indicate that the neuroleptic effect of the phenothiazines (and of certain other groups of preparations with similar structure) are interlinked with their ability to inhibit stimulation of adenylate cyclase by agonists, to bind with the D-1 receptor, and to displace agonists from receptors [7, 9, 10-12].

In this investigation the action of ethmozine and ethacizine and also of typical neuroleptics of the phenothiazine and butyrophenone series on adenylate cyclase in the corpus striatium of the rabbit brain was compared.

EXPERIMENTAL METHOD

A membrane preparation of adenylate cyclase was obtained from the rabbit corpus striatum by a modified method [8]. The corpus striatum and caudate nucleus [1], separated from the remainder of the brain, were homogenized in a Potter—Elvehjem homogenizer in 25 volumes of homogenization medium containing 0.32 M sucrose, 10 mM Tris-HCl, 0.1 mM EDTA, and 1 mM DTTE,

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