Br. J. Pharmacol. (1988), **94**, 871–881

Interactions of benztropine, atropine and ketamine with veratridine-activated sodium channels: effects on membrane depolarization, K^+-efflux and neutrotransmitter amino acid release

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1 The effect of benztropine, atropine and ketamine on veratridine-induced efflux of K^+, membrane depolarization and release of amino acid neurotransmitters was investigated in the preparation of rat brain synaptosomes.

2 All three drugs inhibited in a concentration-dependent manner the processes measured: the most effective compound was benztropine which exhibited an approximate K_d of 2 μM. The inhibition was not competitive in nature.

3 The veratridine titration curves in the presence of drugs were sigmoid with Hill coefficients of about 1.4.

4 At higher concentrations, benztropine, atropine and ketamine blocked uptake of amino acid neurotransmitters into synaptosomes.

5 It is postulated that benztropine, atropine and ketamine interfere with the veratridine-activated influx of sodium into synaptosomes through voltage-dependent channels by acting at the same site as local anaesthetics. Interactions at this site alter allosterically binding and action of veratridine. In addition, at higher concentrations the drugs interact with the carrier proteins for amino acid neurotransmitters and block their transport.

Introduction

It has been known since the early studies of De Elío (1948) that the anticholinoceptor drug, atropine, exhibits pronounced local anaesthetic effects. Iontophoretic application of this compound into mammalian cerebral cortex (Krnjević & Phillis, 1963) has shown it to be a potent depressant of central neurones, consistent with its local anaesthetic properties. Although it has been suggested that atropine, like procaine, has a direct action on the postsynaptic membrane, most likely by preventing increase in sodium permeability (Curtis & Phillis, 1960), the detailed mechanisms of this effect have not been explored to-date. During the course of our studies on the role of plasma membrane transporters in neurotransmitter release (Erecińska *et al.*, 1987) we noted that benztropine, a synthetic molecule composed of atropine and the antihistaminic compound, diphenhydramine, was a powerful inhibitor of veratridine-stimulated efflux of γ-aminobutyric acid (GABA), aspartate and glutamate. Since local anaesthetics are known to interact with voltage-sensitive sodium channels (Catterall, 1987), the object of this investigation was to explore the effects of benztropine and atropine on veratridine-induced increase in permeability of plasma membrane to Na^+, and of its consequences, in preparations of nerve ending particles (synaptosomes) derived from rat brain. These results are compared with those for the dissociative anaesthetic ketamine, which has been reported to interact with the ion channel of the acetylcholine receptor (Maleque *et al.*, 1981).

Methods

Synaptosomes were isolated from fore- and midbrains of male Sprague-Dawley rats (180–220 g) essentially as described by Booth & Clark (1978).

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The final pellet was suspended in a modified Krebs-Henseleit saline (composition, mM: NaCl 140, KCl 5, $NaHCO_3$ 5, $MgSO_4$ 1.3, NaH_2PO_4 1, $CaCl_2$ 1.25) containing 10 mM tris-N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), pH 7.4, and 10 mM glucose as the respiratory substrate.

Measurement of amino acid release

Amino acid release was measured by two methods. In the first, synaptosomes were suspended at 3.9–4.4 mg protein ml^{-1} and preincubated for 10 min at 28 ± 1°C in a shaking water bath. At the end of preincubation, aliquots were withdrawn and pipetted into Erlenmeyer flasks containing appropriate concentrations of various test compounds. After 5 min incubation under the same conditions, 250 µl samples were placed in 400 µl microfuge tubes and the synaptosomes centrifuged rapidly (Beckman microfuge) through a layer of silicone oil (sp. gr. 1.03, General Electric, Waterford, NY). The top layer, which contained the extracellular medium, was pipetted off, acidified with trichloroacetic acid (final concentration 0.1 M) and used for amino acid measurement. The concentrations of amino acids were determined by high pressure liquid chromatography (h.p.l.c) of their *o*-phthaldialdehyde 2-mercaptoethanol derivatives (Jarrett *et al.*, 1986). Preparation of the samples and methods of detection were as described previously (Erecińska *et al.*, 1983). The retention times were (min): aspartate, 2; glutamate, 4; GABA, 11.

In the second procedure, the synaptosomes were suspended at about 10 mg protein ml^{-1} and preincubated for 25 min at 30 ± 1°C in a shaking water bath with 2 µM [^{14}C]-GABA (0.5 µCi ml^{-1}) and 1 µM [^{3}H]-D-aspartate (1 µCi ml^{-1}) plus 1 mM aminooxyacetic acid. At the end of preincubation, aliquots were taken and diluted 10 fold into media containing appropriate concentrations of various test compounds. Samples (200 µl) were withdrawn at 30, 60, 90 and 120 s and centrifuged through silicone oil as above. Radioactivity was then measured in the supernatants in a Delta 300 liquid scintillation counter using Liquiscint LS-121.

Measurement of amino acid uptake

Synaptosomes (about 5 mg protein ml^{-1}) preincubated for 10 min at 28 ± 1°C were diluted 5 fold into media containing various concentrations of test compounds and supplemented with 2 µM [^{14}C]-GABA (0.1 µCi ml^{-1}) and 2 µM [^{3}H]-D-aspartate (0.1 µCi ml^{-1}). Samples (350 µl) were withdrawn at 30, 60 and 90 s and rapidly centrifuged through silicone oil as above. The rates of uptake were calculated by linear regression analysis of the increase in radioactivity in the pellets.

Measurements of extracellular potassium

Synaptosomes were suspended at 4–5 mg protein ml^{-1} in a medium containing 3.0 mM KCl (instead of 5 mM normally present) and placed in a cuvette equipped with a small magnetic stirrer and a K^+-sensitive valinomycin electrode (Pick *et al.*, 1973). Concentration of potassium in the medium was monitored continuously. After 10 min preincubation, various compounds were added as required by the experimental protocol. The electrode was calibrated with a standard solution of KCl at the beginning and end of each experiment.

Determination of transmembrane electrical potential

Synaptosomes preincubated for 10 min at 4–4.5 mg protein ml^{-1} were pipetted into Erlenmeyer flasks containing appropriate concentrations of various test compounds and supplemented with 10 µM [^{14}C]-triphenylmethylphosphonium ([^{14}C]-TPMP) and 2 µM tetraphenylboron. After 15 min incubation at 28 ± 1°C, 250 µl aliquots were taken and centrifuged through silicone oil as above. Transmembrane electrical potentials were calculated from the equilibrium distribution of TPMP and the measured intrasynaptosomal water volume as described by Deutsch & Rafalowska (1979). Preliminary experiments showed that 15 min incubation was sufficient to ensure equilibrium.

Measurement of intrasynaptosomal water volume

Tritiated water and [^{14}C]-polyethylene glycol (mol. wt. 4000) were added to the suspension of preincubated synaptosomes (10 min at 28 ± 1°C) and incubated for 30 s. After centrifugation through silicone oil, the total water volume of the pellet was calculated from the content of ^{3}H whereas the extracellular water was determined from the content of ^{14}C.

Measurement of protein concentration

Protein concentration was determined by the biuret reaction (Gornall *et al.*, 1949) with bovine serum albumin as the standard.

[^{14}C]-GABA, 231 mCi $mmol^{-1}$; [^{3}H]-D-aspartate, 15.0 Ci $mmol^{-1}$, [^{3}H]-water, 0.25 mCi g^{-1} and [^{14}C]-polyethyleneglycol, 0.82 mCi g^{-1} were purchased from New England Nuclear, Boston, MA, U.S.A. [^{14}C]-triphenylmethylphosphonium, 4.8 mCi $mmol^{-1}$ was obtained from Amersham,

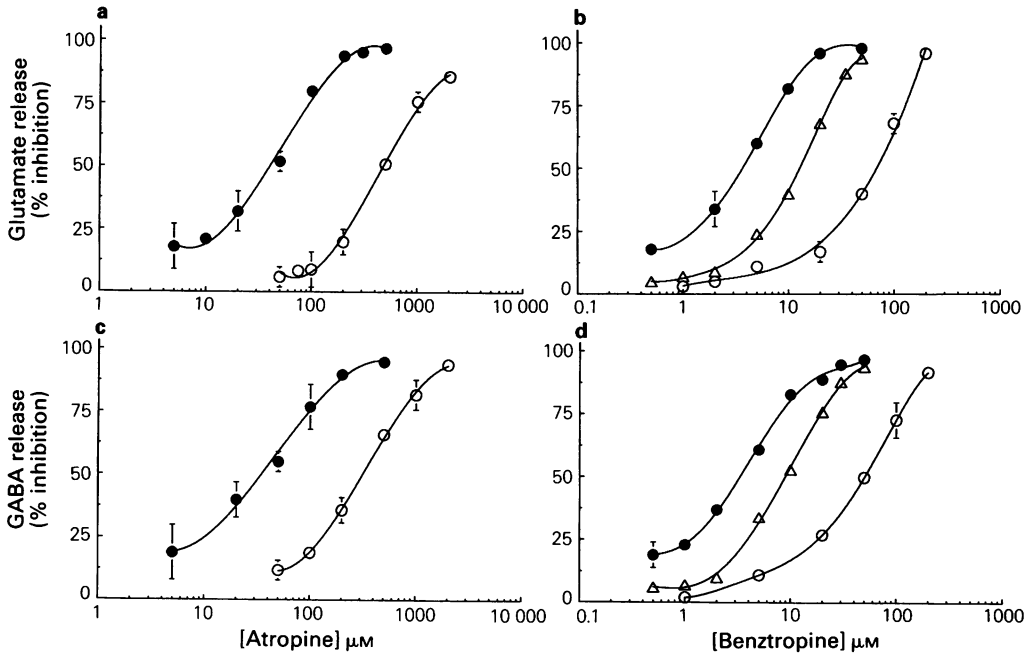

Figure 1 Effect of benztropine and atropine on veratridine-stimulated release of γ-aminobutyric acid (GABA) and glutamate. Release of amino acids were measured by h.p.l.c. as described in the Methods. Veratridine 2.5 μM (●), 10 μM (△) and 50 μM (○). Values are means for 4 (2.5 and 50 μM veratridine) or 2 (10 μM veratridine) experiments; s.d. shown by vertical bars except when less than size of symbol. The absolute amounts of amino acids released into the medium are given in the text.

Arlington Heights, IL, U.S.A., and Liquiscint LS-121 from National Diagnostic, Somerville, NJ, U.S.A. Veratridine, atropine sulphate and benztropine mesylate were from Sigma Chemical Co., St Louis, MO, U.S.A. and ketalar (ketamine hydrochloride) was from Parke-Davies, Morris Plains, NJ, U.S.A.

Results

Inhibition by benztropine and atropine of veratridine-induced release of amino acid neurotransmitters

Addition of veratridine to a suspension of synaptosomes caused concentration-dependent release of GABA, aspartate and glutamate. After 5 min incubation, 2.5 μM alkaloid caused release (per mg of synaptosomal protein) of 0.71 ± 0.11 nmol of GABA, 1.15 ± 0.9 nmol of aspartate and 2.38 ± 0.36 nmol of glutamate. At 10 μM veratridine the respective values were: 1.46 ± 0.22, 2.30 ± 0.11 and 4.47 ± 0.01 nmol and at 50 μM, 2.85 ± 0.55, 3.25 ± 0.22 and 6.08 ± 0.25 nmol (means ± s.d. for 3–5 experiments). Figure 1 shows that when synaptosomes incubated with veratridine were simultaneously exposed to increasing concentrations of either benztropine or atropine an inhibition of amino acid release was observed, which reached 100% for both drugs. It can also be noted that as the concentration of veratridine was raised, larger amounts of the drugs were required to cause similar inhibitions: consequently the titration curves were shifted to the right although they remained parallel. The pattern of responses for the neutral amino acid, GABA, and for the acidic amino acid, glutamate, were the same. Inhibition of aspartate release was essentially identical to that of glutamate and is not shown here.

Benztropine inhibited GABA release with a $K_{0.5}$ of 2 μM, 8 μM and 50 μM at 2.5, 10 and 50 μM veratridine, respectively, while the values for atropine inhibition were 20 μM at 2.5 μM veratridine and 200 μM at 50 μM alkaloid. The $K_{0.5}$ values for the inhibition of glutamate efflux were slightly, but not significantly higher than those for GABA. Since the lowest concentration of veratridine used in these studies, 2.5 μM, was substantially below its K_D (Catterall, 1975; 1980), the $K_{0.5}$ for benztropine and atropine at this alkaloid concentration gives a reasonable approximation of their respective K_D values.

Table 1 Effect of benztropine and atropine on veratridine-induced depolarization of synaptosomal membrane

Drug	*Membrane potential* (mV)	n
None	−74 ± 3	9
Veratridine, 2.5 μM	−61 ± 4	5
Veratridine, 10 μM	−47 ± 1	3
Veratridine, 50 μM	−38 ± 3	5
Veratridine, 2.5 μM + benztropine, 20 μM	−71 ± 2*	4
Veratridine, 10 μM + benztropine, 20 μM	−60 ± 3	3
Veratridine, 50 μM + benztropine, 20 μM	−52 ± 2	4
Veratridine, 50 μM + benztropine, 100 μM	−65 ± 3	3
Veratridine, 2.5 μM + atropine, 100 μM	−73 ± 2*	3
Veratridine, 50 μM + atropine, 500 μM	−54 ± 2	3
KCl, 40 mM	−42 ± 6	4
KCl, 40 mM + benztropine, 100 μM	−40 ± 4	4

Membrane potentials were calculated from the distribution of triphenylmethylphosphonium as given in the Methods. Values represent means ± s.d. for the number of experiments indicated. Statistical analysis of the data was performed by use of two-tailed Student's *t* test. The following results were obtained: (a) all values were significantly different from the control (no drugs) at the level of $P < 0.001$ except those designated with an asterisk which were not significant; (b) values with benztropine (or atropine) + veratridine were significantly different from those at the same veratridine concentration at the level of $P < 0.001$ except those with 2.5 μM veratridine for which P was < 0.005.

This yields figures of 2 μM and 20 μM for the two drugs, respectively. Scopolamine also blocked veratridine-induced amino acid efflux although at concentrations about 4–6 fold higher than atropine. In view of the high doses required for inhibition, the effect of this compound was not investigated further.

Benztropine and atropine also inhibited release of GABA and aspartate from synaptosomes preloaded by incubation with the respective radioactive amino acids. The release caused by 2.5 μM veratridine was blocked completely by either 20 μM benztropine or 200 μM atropine whereas that induced by 50 μM alkaloid required 10 fold higher concentrations of the drugs. The effect was very rapid and was already fully developed in the initial 30 s.

In some experiments, incubations with veratridine, with and without benztropine and atropine were carried out for 30 min. Although the release of amino acids was about 10–15% greater, the same inhibition by the two drugs was observed.

Inhibition by benztropine and atropine of veratridine-induced membrane depolarization and potassium efflux

Simultaneous inhibition of both GABA and acidic amino acid efflux suggests that a common mechanism may be involved in the phenomena observed. Since veratridine is known to induce depolarization of the plasma membrane through activation of the voltage-dependent sodium channels (Ulbricht, 1969; Catterall, 1975; 1980), the effect of benztropine and atropine on synaptosomal membrane potential was investigated. This was done in two ways. In the first, transmembrane electrical potential was measured directly from the distribution of the lipophilic cation TPMP. In the second, changes in extrasynaptosomal potassium were followed with a K^+-sensitive electrode. Since in synaptosomes, membrane potential is the potassium diffusion potential (Blaustein & Goldring, 1975; Deutsch & Rafalowska, 1979), loss of this cation from the nerve endings and a simultaneous increase in its concentration in the external environment is indicative of depolarization.

Table 1 shows that addition of increasing concentrations of veratridine caused progressive depolarization of the synaptosomal membrane. Consistent with the data in Figure 1, benztropine and atropine inhibited veratridine-induced decrease in membrane potential. At the lowest alkaloid concentration used, a smaller amount of either drug was required to prevent depolarization completely whereas with higher concentrations of veratridine, larger amounts of benztropine or atropine were necessary to obtain the same degree of inhibition. Figure 2 illustrates this pattern more clearly: titrations with benztropine at two veratridine concentrations were remarkably similar to the curves shown in Figure 1. The plot at 50 μM veratridine was again shifted to the right with respect to that at 2.5 μM alkaloid, but similar in shape.

Neither benztropine (up to 200 μM) nor atropine (up to 2 mM) alone had any effect on the distribution of TPMP which suggests that these drugs do not

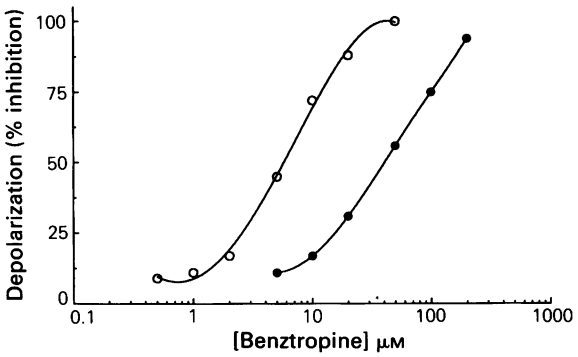

Figure 2 Titration with benztropine of depolarization of synaptosomal membrane caused by veratridine. Control values of membrane potential and those obtained with 2.5 (●) and 50 (○) μM veratridine were identical to the figures given in Table 1. Plots represent means of two experiments which were within 5% of each other.

interfere with transport of this cation across the synaptosomal membrane. Moreover, these results indicate that at the concentrations used, neither compound affects the sodium/potassium ATPase because inhibition of this enzyme would result in membrane depolarization. It should also be pointed out that the absolute values for membrane potential appear somewhat high, higher than those determined from the distribution of either potassium or radioactive rubidium, because they were not corrected for the binding of TPMP. However, as shown by previous authors (Deutsch & Rafalowska, 1979; Creveling *et al.*, 1980; 1983), this binding of the lipophilic cation does not affect the magnitude of changes in membrane potential during depolarization with either veratridine or high potassium.

Essentially the same behaviour as that seen from TPMP distribution was observed when external potassium concentrations were followed (Figures 3 and 4). Loss of potassium from synaptosomes was dependent on the amount of veratridine added and could be inhibited, in a concentration-dependent manner, by benztropine: the final outcome was always the result of the interplay between the veratridine and benztropine concentrations. When the drug was added at the appropriate concentration a few seconds before the alkaloid, no release of potassium was seen. On the other hand, when benztropine was added after the veratridine-induced efflux of potassium had stabilized, it caused very rapid disappearance of the cation from the medium.

Effect of veratridine on membrane depolarization and amino acid release in the presence of benztropine

The parallel, right-handed shift in the titration curves shown in Figures 1 and 2 might suggest that benztropine and atropine are competitive inhibitors of veratridine action. To gain insight into the mechanisms of this inhibition, titrations with veratridine were carried out at various constant bentropine concentrations. The results of these experiments are shown for depolarization in Figure 5 and for GABA release in Figure 6. It can be seen that titration with veratridine in the absence of drugs showed a monotonic rise with a $K_{0.5}$ for the alkaloid of about 10 μM for both processes. With benztropine present, the curves were shifted to the right and the maximum response was depressed progressively with increasing drug concentration. This inability of veratridine to reverse completely the effect of benztropine indicates that these compounds act at different sites, i.e., inhibition by benztropine is not competitive in nature. Moreover, the titration curves with benztropine present appeared sigmoid in shape. Hill plots of the results obtained for GABA release (Figure 7) showed that the titration with veratridine had a slope of 1, as expected (Ulbricht, 1969; Catterall, 1975), whereas those with either 20 or 50 μM benztropine had slopes of 1.4–1.43. Similar results were obtained with atropine (not shown). Such behaviour is suggestive of cooperative interactions between the veratridine and benztropine (atropine) binding sites.

Role of membrane potential in benztropine-induced inhibition of veratridine-activated amino acid release

Since veratridine-induced increase in Na^+ permeability is accompanied by depolarization of synaptosomal plasma membrane, it was considered necessary to determine whether depolarization itself, at a constant alkaloid concentration, alters the response to benztropine or atropine. For this reason, release of amino acids was measured in synaptosomes treated with 2.5 μM veratridine and simultaneously depolarized by addition of 20 mM KCl. The results of a typical experiment are shown in Figure 8. It can be seen that the plot of percentage inhibition of GABA release against benztropine concentration in the presence of veratridine plus KCl showed a $K_{0.5}$ for benztropine of 6 μM and was remarkably similar to the curves displayed in Figure 1 and obtained with veratridine only. When the background release caused by KCl was subtracted from that caused by veratridine plus KCl, the curve denoted as 'calculated' was obtained. In essence, it represents the effect of benztropine on veratridine-induced release of GABA in the presence of membrane depolarization. The $K_{0.5}$ for benztropine derived from this plot is about 2.5 μM which is essentially the same as that of 2 μM obtained from Figure 1. This suggests that membrane potential has no effect on benztropine interaction with synaptosomal membrane.

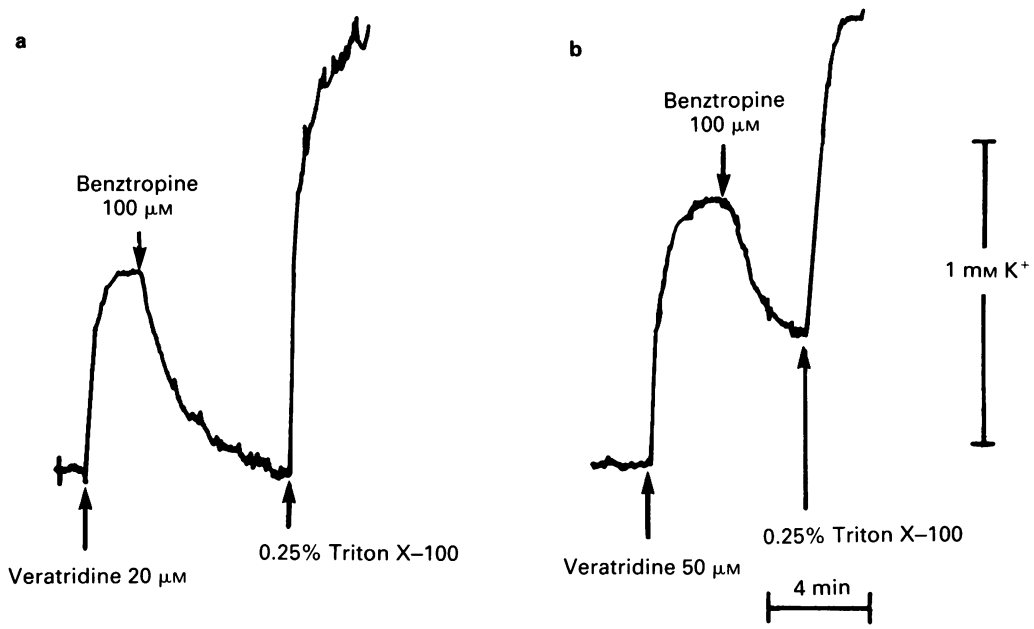

Figure 3 Effect of constant benztropine concentration on $[K^+]_e$ in synaptosomal suspension treated with 20 μM (a) and 50 μM (b) veratridine. Measurements of potassium were carried out as described in the Methods. Arrows indicate additions of the compounds of interest. At the end of each experiment, triton X-100 was added to destroy the synaptosomal membrane and allow calculations of the total content of potassium in the preparation. The increase in the medium $[K^+]$ after complete rupture of synaptosomes was 1.2–1.5 mM which at 4–5 mg protein ml^{-1} translates into the value of about 300 nmol of potassium per mg of protein.

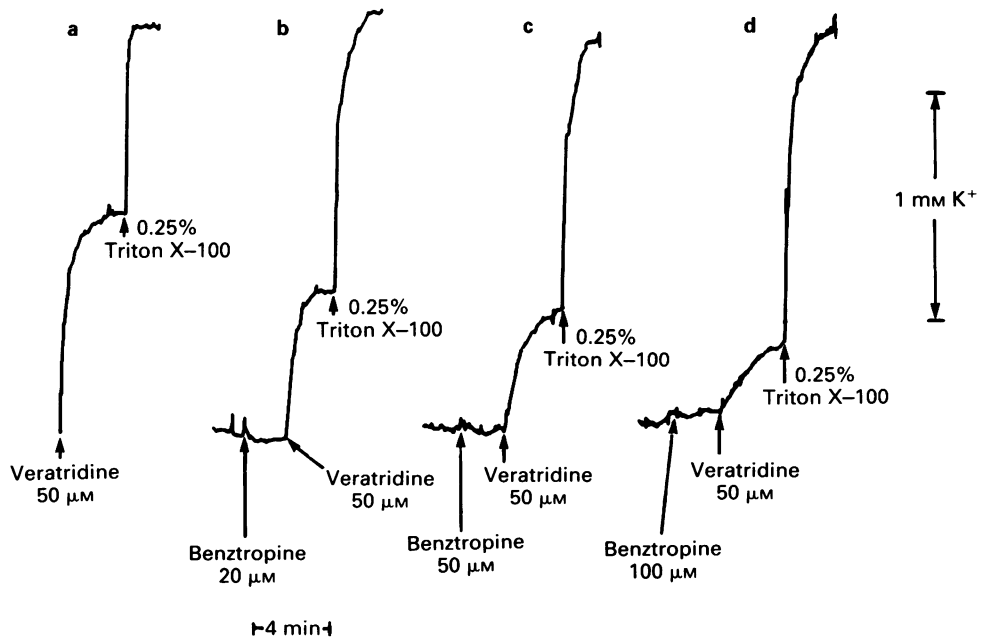

Figure 4 Titration with benztropine of veratridine-induced K^+-leakage: conditions are given in the Methods and in the legend to Figure 3.

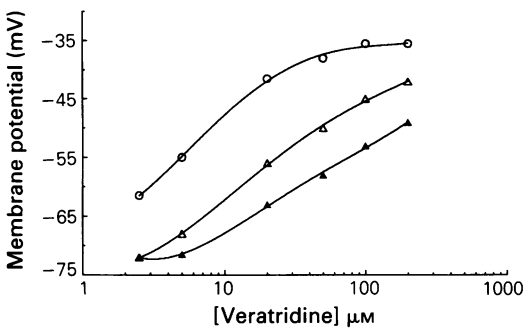

Figure 5 Effect of veratridine on membrane potential of synaptosomes incubated with and without benztropine: 20 μM (△) and 50 μM (▲) benztropine; without benztropine (○). Experimental conditions are given in the Methods. A typical experiment carried out on the same synaptosomal preparation is presented.

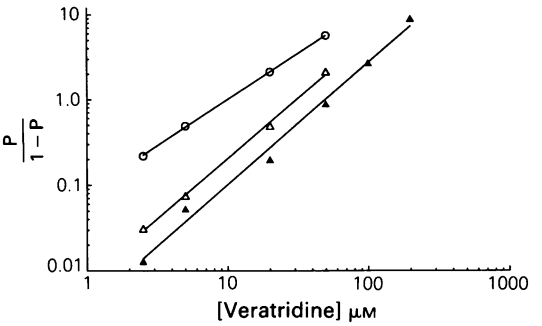

Figure 7 Hill plot for the veratridine-stimulated γ-aminobutyric acid (GABA) release with and without benztropine: 20 μM (△) and 50 μM (▲) benztropine; without benztropine (○). The results from Figure 6 were used in the plot. Linear regression analysis of the data yielded the following results: without benztropine, slope 1.03, $r = 1.00$; with 20 μM benztropine, slope 1.40, $r = 0.999$; with 50 μM benztropine, slope, 1.43, $r = 0.995$.

It is worth noting that benztropine somewhat inhibited KCl-stimulated release of amino acids. At the same drug concentration the effect was, however, much smaller than that on veratridine-induced changes. Benztropine at 50 μM blocked KCl-stimulated release by about 25%, whereas it virtually eliminated that caused by veratridine. By contrast, neither benztropine (100 μM) nor atropine (1 mM) had any influence on depolarization caused by 40 mM KCl. With KCl, the membrane potential calculated from TPMP distribution was -42 ± 5 mV and values of -32 to -42 mV were obtained with the drugs present.

Influence of ketamine on amino acid release and membrane depolarization induced by veratridine

Figure 9 shows that ketamine inhibited veratridine-induced release of GABA with an approximate K_D of 100 μM. It also prevented veratridine-induced depolarization of synaptosomal membrane (not shown). Moreover, like the other two drugs studied above, ketamine was without effect on KCl-induced decrease in membrane potential although at higher

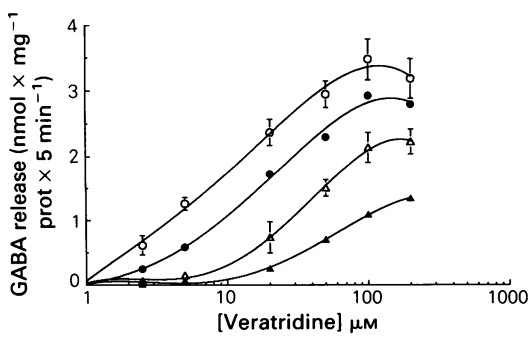

Figure 6 Effect of veratridine on γ-aminobutyric acid (GABA) release from synaptosomes incubated with and without benztropine: 5 μM (●), 20 μM (△) and 50 μM (▲) benztropine; without benztropine (○), GABA was measured by h.p.l.c. as given in the Methods. Values are means for 3 (0 and 20 μM benztropine) or 2 (5 and 50 μM benztropine) experiments; s.d. shown by vertical bars.

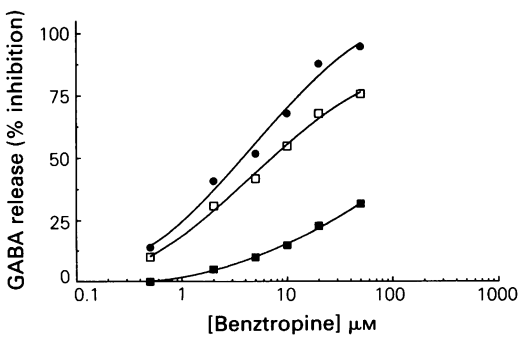

Figure 8 Influence of depolarization with KCl on inhibition by benztropine of veratridine-stimulated release of γ-aminobutyric acid (GABA): 20 mM KCl (■); 20 mM KCl plus 2.5 μM veratridine (□); 2.5 μM veratridine (●). The 'calculated' curve (●) was obtained by subtracting absolute values of GABA (nmol $5\,min^{-1}$) released by KCl from those secreted by veratridine + KCl and then determining the percentage inhibition. Data points represent means of 2 experiments which were within 5% of each other.

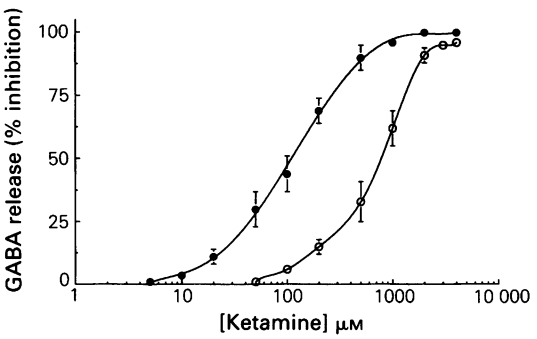

Figure 9 Inhibition by ketamine of veratridine-induced γ-aminobutyric acid (GABA) release: 2.5 μM (●) and 50 μM (○) veratridine. Data points represent means for 3 experiments; s.d. shown by vertical bars.

concentrations (>500 μM) it blocked KCl-evoked efflux of amino acids to some degree. The concentrations of ketamine required to reach the same effect were 5 fold larger than those of atropine and 50 fold larger than those of benztropine. However, the pattern of responses was the same.

Effect of benztropine, atropine and ketamine on GABA and acidic amino acid uptake

The effects of benztropine, atropine and ketamine on GABA and D-aspartate uptake are summarized in Table 2. All three drugs inhibited, to some extent, transport of amino acids into synaptosomes. The concentrations required to attain 50% inhibition of GABA uptake were about 100 μM for benztropine, slightly more than 1 mM for atropine and about 2 mM for ketamine. It is interesting that transport of D-aspartate, a representative of the acidic amino acid system (DeFeudis, 1975; Johnson, 1978; Erecińska, 1987), was much less sensitive to the effects of these drugs. Benztropine at 100 μM blocked D-aspartate uptake by 24% (Table 2), whereas 2 mM atropine and 5 mM ketamine were required to attain the same degree of inhibition (data now shown).

Discussion

The results presented in this paper show that benztropine, atropine and ketamine block veratridine-induced membrane depolarization, potassium efflux and release of amino acid neurotransmitters. All three processes are inhibited by approximately the same concentration of each drug which suggests that a common underlying cause is involved. Although we did not measure directly the influx of sodium, owing to inherent difficulties in making such measurements in synaptosomes, the most likely explanation, consistent with the data, is inhibition of the alkaloid-activated influx of this cation through voltage-dependent channels. The question then arises as to how these compounds interact with the sodium channel? The noncompetitive character of inhibition of veratridine action and pronounced sigmoid properties of alkaloid titration curves in the presence of these drugs are strikingly similar to the pattern of behaviour shown by local anaesthetics (Catterall, 1975; 1977; 1980; 1981; Creveling *et al.*, 1983). Hence, in agreement with the postulate of Catterall, we would like to suggest that benztropine, atropine and ketamine act at the same site(s) on the Na^+ channels as local anaesthetics and that interactions at these site(s) alter, allosterically, binding and action of veratridine at site 2 (Catterall, 1980; 1987). Of the three compounds tested, the most effective in inhibiting activation of sodium channels is benztropine whose K_D of 2–3 μM lies within the range of values

Table 2 Influence of benztropine, atropine and ketamine on γ-aminobutyric acid (GABA) and D-aspartate uptake by rat brain synaptosomes

Drug	Concentration (μM)	GABA uptake (% control)	P	D-aspartate uptake (% control)	P
Benztropine	20	69 ± 3	<0.001	90 ± 2	<0.001
	100	44 ± 4	<0.001	76 ± 4	<0.001
Atropine	200	93 ± 2	<0.001	98 ± 9	NS
	1000	69 ± 4	<0.001	96 ± 2	<0.05
Ketamine	400	92 ± 4	<0.001	116 ± 12	<0.05
	2000	53 ± 5	<0.001	101 ± 12	NS

Results represent means ± s.d. for 3 experiments. Rates of uptake of 2 μM [^{14}C]-GABA and 2 μM [^{3}H]-D-aspartate were measured as described in the Methods. Control (100%) values were: 0.48 ± 0.03 nmol min^{-1} mg^{-1} protein for GABA and 0.95 ± 0.01 nmol min^{-1} mg^{-1} protein for D-aspartate. Two tailed Student's *t* test was used for statistical evaluation of the data.

found for the most potent local anaesthetics (Postma & Catterall, 1984).

Although the three processes followed in this work, membrane depolarization, potassium efflux and amino acid release are treated as independent phenomena, they are intimately linked to each other. Depolarization with veratridine necessarily involves loss of potassium from synaptosomes because membrane potential in the nerve endings is the K^+-diffusion potential (Blaustein & Goldring, 1975; Deutsch & Rafalowska 1979). (It is worth noting that in the presence of the alkaloid, the membrane potential is prominently a function of the sodium diffusion potential as well as the K^+ diffusion potential because veratridine increases g_{Na}.) Moreover, depolarization opens calcium channels and causes influx of this cation which then triggers secretion of neurotransmitters. Hence, any interference with the primary cause, i.e., that which alters membrane potential, would be expected to involve changes in the other two processes.

It is interesting to note that the antiparkinsonian drug, benztropine, interacts with sodium channels with a K_D that is of the same order (0.4–4 μM) as the K_i for inhibition of catecholamine transport in the central nervous system (Horn *et al.*, 1970). Whether or not such interactions contribute to the effects, or side effects, of the drug in patients treated with benztropine, remains to be established.

In addition to affecting the Na^+ channels, all three drugs inhibit uptake of neurotransmitter amino acids into synaptosomes. This appears to be via an independent site of action for these compounds, most likely on the carrier proteins themselves. A general, membrane-fluidizing effect (Sweet *et al.*, 1987) is unlikely because other plasma membrane functions such as K^+-permeability, or the activity of the Na^+/K^+ ATPase, are unaffected. However, in the action on the transport of amino acid neurotransmitters certain selectivity is observed: at the same drug concentration, GABA uptake is inhibited more than that of the acidic amino acid, D-aspartate. Although transport of GABA and of the acidic amino acids occurs on separate carrier proteins, in both cases amino acids are taken up as complexes with sodium ion (Martin, 1976; Nelson & Blaustein, 1982; Pastuszko *et al.*, 1982; Kanner, 1983). It is tempting to speculate that binding of benztropine, atropine and ketamine alters, somehow, interactions of the transporters with sodium. The differences in susceptibility of the two transporters to these drugs could be linked, in some manner, to the differences in their K_m for sodium: GABA transport exhibits an apparent K_m for Na^+ of about 70 mM (Blaustein & King, 1976; Erecińska, 1987) whereas the acidic amino acid transport shows a K_m of about 15 mM (Balcar & Johnston, 1973; Erecińska, 1987). Consistent with the direct inhibition of the transporters is the observation that high KCl-induced release is also sensitive to inhibition by these drugs while membrane depolarization is not. It is well established that in synaptosomes a certain portion of stimulated release occurs through the reversal of the uptake pathway and involves the carrier proteins (Haycock *et al.*, 1978; Nelson & Blaustein, 1982; Sihra *et al.*, 1984; Erecińska, 1987). Studies are currently under way to elucidate the possible mechanism(s) of this inhibition of neurotransmitter amino acid transport. It is worth mentioning that lignocaine has been shown to inhibit Na-linked transport of various amino acids in renal brush-border membranes (Schell & Wright, 1987).

Ketamine, a congener of phencyclidine, and a dissociative anaesthetic, exhibits a number of apparently independent properties: it interacts with the ionic channel of the acetylcholine receptor at the neuromuscular junction (Maleque *et al.*, 1981), it inhibits N-methyl-D-aspartate activated receptors in the central nervous system (Anis *et al.*, 1983; Thomson *et al.*, 1985), it blocks calcium-induced contractions in depolarized uterus (Calixto & Loch, 1985) and it prevents nicotinic agonist-induced secretion of catecholamines from cultured bovine adrenal chromaffin cells (Purifoy & Holz, 1984). Two more effects of the drug are reported here: that on the sodium channel and on the amino acid transport systems. The K_D for interaction of ketamine with a sodium channel, about 100 μM, is only slightly higher than the anaesthetic dose of the drug (70 μM). Hence, it is possible that inhibition of sodium permeability may also contribute to this already complex spectrum of ketamine effects in the central nervous system. On the other hand, inhibition of GABA uptake occurs at higher concentrations of the drug (millimolar, Table 2), in agreement with the finding of Minchin (1981) that 100 μM ketamine had almost no influence on this process.

The inhibition of release of amino acid neurotransmitters which follows interaction of benztropine with the Na^+ channels, shown so clearly in this work, raises the question of whether this compound could be useful in the treatment of hypoxia and ischaemia. In these pathological states, activation of sodium permeability through depolarization triggers release of glutamate and aspartate, two amino acid neurotransmitters that have been implicated as being instrumental in brain damage caused by limitations in oxygen supply. Moreover, persistent and massive depolarization of the neuronal membrane reverses the mode of operation of the transporter proteins and favours release of amino acids. Since both the sodium channels and the transporters are targets for benztropine action, there are at least theoretical grounds for proposing that the use of this drug

should ameliorate the damage. In agreement with this suggestion, it has been reported that intravenous lignocaine significantly reduced neural decrement and increased the recovery of neural function after acute cerebral ischaemia induced in cats by air embolism (Evans et al., 1984).

This work was supported by a grant NS10939 from the National Institutes of Health, U.S.A.

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(Received October 8, 1987
Accepted February 18, 1988)