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Phenothiazines and Haloperidol Block Ca-Activated K Channels in Rat Forebrain Synaptosomes

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SUMMARY

Ca-dependent, K-stimulated ^{86}Rb efflux, a measure of Ca-activated K conductance in rat brain synaptosomes, was blocked by phenothiazines and haloperidol. Micromolar concentrations of the phenothiazines, fluphenazine and trifluoperazine, and haloperidol, a non-phenothiazine antipsychotic and calmodulin antagonist, selectively inhibited the Ca-activated K channels. The IC_{50} values of all three agents for inhibition of the Ca-activated K channels was on the order of 0.5–1 μM. Measurements of K-stimulated ^{45}Ca uptake indicated that the effects of these agents on Ca-activated K channels was not due to inhibition of Ca influx through voltage-gated Ca channels. Sulpiride, a potent antipsychotic with weak anti-calmodulin activity, was a relatively weak inhibitor of Ca-activated K channels. Calmidazolium (compound R-24571) and W7, two non-phenothiazine calmodulin antagonists, did not selectively inhibit Ca-activated K channels. Biphasic dose response curves for inhibition of the Ca-dependent, K-stimulated ^{86}Rb efflux by the phenothiazines raise the possibility that there may be two kinds of Ca-activated K channels in rat brain presynaptic terminals, with different sensitivities to the phenothiazines. These results demonstrate that two phenothiazines and haloperidol are potent and relatively selective inhibitors of Ca-activated K channels in nerve endings. This inhibition does not appear to be mediated by calmodulin or by dopamine receptors.

Calcium-activated potassium channels are found in a wide variety of excitable cells including neurons (1), muscle (2), and secretory cells (3). In general, a rise in the intracellular ionized calcium concentration activates these channels; this causes hyperpolarization, a reduction in other voltage-gated conductances, and a lower level of excitability. In the vertebrate nervous system there are probably at least two distinct types of Ca-activated K conductances (4). These conductances may accelerate the repolarization of the action potential and reduce the firing rate of neurons ("accomodation") during sustained stimulation (5, 6). Because of their influence on firing frequency, Ca-activated K conductances may also be involved in epileptogenesis (7). Ca-activated K channels in nonexcitable cells may regulate intracellular osmolarity and electrolyte concentrations (8, 9).

Intracellular free Ca also serves as a "second messenger" in the control of numerous other functions in a variety of cell types. In many instances the action of Ca is mediated by the Ca-binding protein, calmodulin (e.g., Refs. 10–12). This protein, which is found in many mammalian tissues, including the brain, has been well characterized (13). Calmodulin is present in high concentration (approximately 1% of total protein) in the presynaptic nerve endings of the CNS (14). Although calmodulin presumably mediates a number of Ca-dependent activities in the CNS, it is not known whether calmodulin regulates Ca-activated K conductances in CNS neurons. This possibility was examined in the present study. If calmodulin is involved in the control of Ca-activated K conductances, it must be very tightly bound to the channels because several investigators have reported that Ca directly gates Ca-activated K channels in membrane patches or planar lipid bilayers in the absence of exogenous calmodulin (2, 15, 16).

In the past, ion channels of nerve endings eluded careful investigation because traditional electrophysiological techniques are inadequate for studying very small structures (<1 μm diameter). However, the development of new ion flux techniques has recently enabled detailed study of Na channels (17, 18), Ca channels (19, 20), and various K channels (21, 22) in isolated nerve endings (synaptosomes). Recent reports from this laboratory demonstrate that rat forebrain synaptosomes exhibit at least four different classes of K channels (see Fig. 1, under Materials and Methods): 1) a non-inactivating K conductance which is open under resting conditions; 2) a voltage-

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ABBREVIATIONS: CNS, central nervous system; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; FLU, fluphenazine; PRO, promethazine; TEA, tetraethylammonium chloride; TFP, trifluoperazine; W7, *N*-(6-aminohexyl)-5-chloro-1-naphthenesulfonamide; AHP, afterhyperpolarization.

gated, rapidly inactivating K conductance; 3) a voltage-gated, non-inactivating K conductance; and 4) a Ca- and voltage-gated K conductance. The pharmacological properties of these channels and their relationship to those observed using electrophysiological techniques are described elsewhere (21, 22).

In this study we used a pharmacological approach to investigate the possibility that calmodulin might be involved in the gating of Ca-activated K channels. Several agents known to inhibit the action of calmodulin were tested. If calmodulin mediates the Ca-dependent activation of these channels, the inhibition of Ca-dependent ^{86}Rb efflux by these drugs should parallel their anticalmodulin activity. In contrast, however, we found that two phenothiazines and haloperidol, but not more selective anticalmodulin agents, were potent inhibitors of Ca-dependent ^{86}Rb efflux from synaptosomes. A preliminary report of these data has appeared in abstract form (23).

Materials and Methods

Synaptosome ^{86}Rb fluxes were measured as described previously (21). Briefly, rat forebrain synaptosomes were prepared according to the procedure of Hajos (24), as modified by Krueger *et al.* (25). Following sucrose density gradient centrifugation, synaptosomes were slowly equilibrated with a physiological salt solution ("5K," see Table 1 for composition). Synaptosomes (protein concentration approximately 40 mg/ml) were then incubated in 5K containing ^{86}Rb (10–20 μCi/ml) for 30 min at 30°.

Following ^{86}Rb loading, an aliquot of tracer-labeled synaptosomes (60 μl) was pipetted onto a glass fiber filter (Schleicher & Schuell, no. 25) and extracellular tracer was removed with a wash solution (Table 1). The wash solution contained the test drugs or vehicle, where appropriate, in order to preexpose the synaptosomes to these drugs for 12–15 sec before the measurement of ^{86}Rb efflux. The wash solution was removed by vacuum filtration and the synaptosomes were then exposed to 0.5 ml of an efflux solution. For time course experiments the following efflux solutions were used: 5K, high K (50K or 100K), or high K + Ca (50K/Ca or 100K/Ca) (see Table 1 for compositions); test drugs or vehicle were also included in the appropriate efflux solutions (see Results). For dose response experiments, 100K or 100K/Ca efflux solutions containing varying concentrations of test drugs (see Results) were used. Efflux incubations were carried out for 1–5 sec (timed with a metronome) at 30°. The incubations were terminated with stop solution (Table 1), and the synaptosome suspensions were rapidly filtered. The ^{86}Rb retained on the filters (containing the trapped synaptosomes) and the ^{86}Rb in the effluent solutions (filtrate) were both determined (as cpm) by liquid scintillation spectrometry. The percentage of total content of radioactivity released during efflux incubation was then calculated as:

$$^{86}\text{Rb Efflux (\% of content)} = \frac{\text{(cpm effluent)}}{\text{(cpm filter)} + \text{(cpm effluent)}}$$

In each experiment the efflux for each condition was measured in quadruplicate.

Fig. 1 illustrates the components of synaptosome ^{86}Rb efflux, as previously characterized by Bartschat and Blaustein (21, 22). The rate of ^{86}Rb loss into 5K solution corresponds to the resting K conductance; when this line (Fig. 1, *solid circles*) is extrapolated back to the origin, the residual tracer in the effluent (usually about 8–12% of the original tracer content) can be attributed to incomplete washing and to delays in initiating the efflux after washing away the residual extracellular ^{86}Rb from the loading solution (21). Incubation in high K (Ca-free 100K; Fig. 1, *solid squares*), which should depolarize the terminals by about 50 mV (21), markedly stimulates the Rb efflux: there is an increase in the zero time intercept and in the slope of the line. The *dashed line* in Fig. 1 corresponds to the expected early time course of the K-stimulated Rb efflux; thus, the K-stimulated efflux can be divided into two components, one of which rapidly inactivates. When the incubation solution is high K + Ca (100K/Ca; Fig. 1, *solid triangles*), there is a further stimulation of ^{86}Rb efflux: the Ca-dependent, K-stimulated Rb efflux, which corresponds to Rb movement through Ca-activated K channels (22). This Ca-dependent component of the ^{86}Rb efflux was selectively blocked by partially purified charybdotoxin (26), a known blocker of the large conductance, TEA-sensitive, Ca-activated K channel (27).

Synaptosome ^{45}Ca uptake was also determined essentially as described (19). Following equilibration of synaptosomes in 5K solution, an aliquot of synaptosomes (20 μl) was diluted (1:10) with 5K or 100K uptake solution containing 100 μM $CaCl_2$, varying concentrations of drug or vehicle (see Results), and 3–4 μCi/ml ^{45}Ca. Uptake was termi-

TABLE 1
Composition of solutions

Solute	5K	100K[a]	100K/Ca[a]	Wash Solution[b]	Stop Solution	EGTA Solution
			mM			
NaCl	145	50	50	145		145
KCl	5	100	100	5		5
RbCl	0.1	0.1	0.1	0.1	0.1	
$MgCl_2$	2	2	1	2	10	2
Glucose	10	10	10	10		10
NaH_2PO_4	0.5	0.5	0.5	0.5		0.5
HEPES[c,d]	10	10	10	10	10	10
$CaCl_2$			1			
TEA					145	
TBA[c]					5	
$NiCl_2$					10	
EGTA						10

[a] In some experiments 50K (or 50K/Ca) was used instead of 100K (or 100K/Ca). These solutions were similar to the respective 100K solutions except that the KCl was reduced to 50 mM, and the K was replaced, mol-for-mol, by Na.
[b] Also contained 1 mg/ml bovine serum albumin.
[c] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TBA, tetrabutylammonium chloride.
[d] Solutions were adjusted to pH 7.4 at room temperature with NaOH.

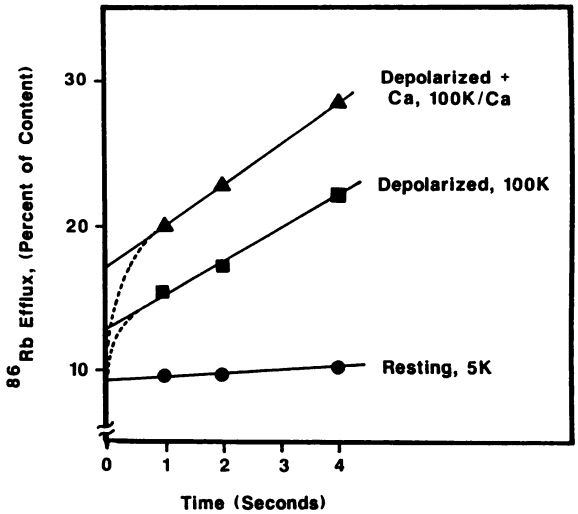

Fig. 1. Time course of ^{86}Rb efflux from synaptosomes: effects of K depolarization and Ca (see Refs. 21 and 22). Data are from a representative experiment. ^{86}Rb efflux was measured for 1–4 sec in the presence of 5K (●), 100K (■), and 100K/Ca (▲) efflux solutions (see Table 1 for composition). The *ordinate* scale is defined under Materials and Methods. ——, linear regression lines. The efflux at times shorter than 1 sec was not measured; – – – approximates the predicted time course of efflux during the initial sec. The curvilinear relationships correspond to rapid inactivation of some components of the efflux in the two K-rich solutions. The Ca-dependent ^{86}Rb efflux is the difference between ▲ and ■; this efflux component corresponds to Rb efflux through Ca-activated K channels (see Ref. 22).

nated after 2 sec by addition of 5 ml of EGTA solution (Table 1), and the suspension was immediately filtered on Whatman GF/B filters.

All salts were reagent grade. TFP, PRO, haloperidol, sulpiride, W7, and calmidazolium (compound R24571) were obtained from Sigma Chemical Co. (St. Louis, MO). FLU was obtained from E. R. Squibb & Sons (Princeton, NJ).

Stock solutions of the phenothiazines and haloperidol were always prepared fresh in 0.01 N HCl. Stock sulpiride solutions were prepared in 0.1 M acetic acid. Calmidazolium was prepared in dimethyl sulfoxide, and W7 was prepared in ethanol. In the experiments with calmidozolium and W7, appropriate dilutions of dimethyl sulfoxide and ethanol, respectively, were included in the control solutions; these solvents had no effect on the control ^{86}Rb efflux.

Results

The effects of phenothiazines on ^{86}Rb efflux and ^{45}Ca uptake in synaptosomes. Phenothiazines are a class of antipsychotic agents that act as dopamine receptor antagonists (Table 2). This antagonism is believed to impart their clinical efficacy (29). At much higher concentrations than those that block dopamine receptors, the phenothiazines also exhibit anticalmodulin activity (Table 2). To determine whether calmodulin is involved in mediating the action of Ca on Ca-activated K channels, we tested the effects of FLU (1 μM) and TFP (1 μM) on ^{86}Rb efflux from synaptosomes. These data (Fig. 2) indicate that the two phenothiazines selectively inhibit the Ca-dependent fraction of ^{86}Rb efflux at this concentration. Low concentrations of TFP and FLU also caused a small reduction in the efflux into 5K medium; this was due primarily to a reduction in the time-independent ("background") loss of ^{86}Rb. They had no effect on the voltage-gated components of the ^{86}Rb efflux (i.e., the components activated by raising external K in the absence of Ca; see Fig. 1). At higher concentrations,

TABLE 2

Comparison of the anticalmodulin, antidopaminergic, and Ca-dependent K channel-blocking properties of several antipsychotic agents and calmodulin antagonists

Drug	Anticalmodulin activity IC_{50}	Antidopaminergic activity K_D (^{3}H-Haloperidol displacement)	Ca-activated K channel block IC_{50}
		nM	
FLU	6,000–50,000[a,b]	1–3[c]	1,500[d]
TFP	6,000–50,000[a,b]	3–5[c]	500[d]
PRO	>100,000[e]	830[f]	>50,000[g]
Haloperidol	2,500–60,000[e]	1[c]	500[g]
Sulpiride	>100,000[e]	30–80[c]	>10,000[g]
W7	30,000[h]	ND[i]	>5,000[j]
Calmidazolium	10–5,000[k]	ND	>5,000[j]

[a] Data from Ref. 31.
[b] Data from Ref. 42.
[c] Data from Ref. 29.
[d] Block of Ca-dependent ^{86}Rb efflux in synaptosomes. The block appeared to be biphasic: the values shown are the approximate drug concentrations for 50% inhibition of the entire Ca-dependent component of ^{86}Rb efflux. These agents appeared to block about 30–40% of the efflux with high affinity (IC_{50} = 20–30 nM) and the remainder with lower affinity (IC_{50} = 1000–3000 nM). See Fig. 4 and related text.
[e] Data from Ref. 43.
[f] Data from Ref. 44.
[g] Block of Ca-dependent ^{86}Rb efflux in synaptosomes (see text).
[h] Data from Ref. 45.
[i] ND, not determined.
[j] W7 (5000 nM) and calmidazolium had no effect on Ca-dependent ^{86}Rb efflux; a higher concentration of W7 inhibited all components of ^{86}Rb efflux nonselectively (see text).
[k] Data from Ref. 46.

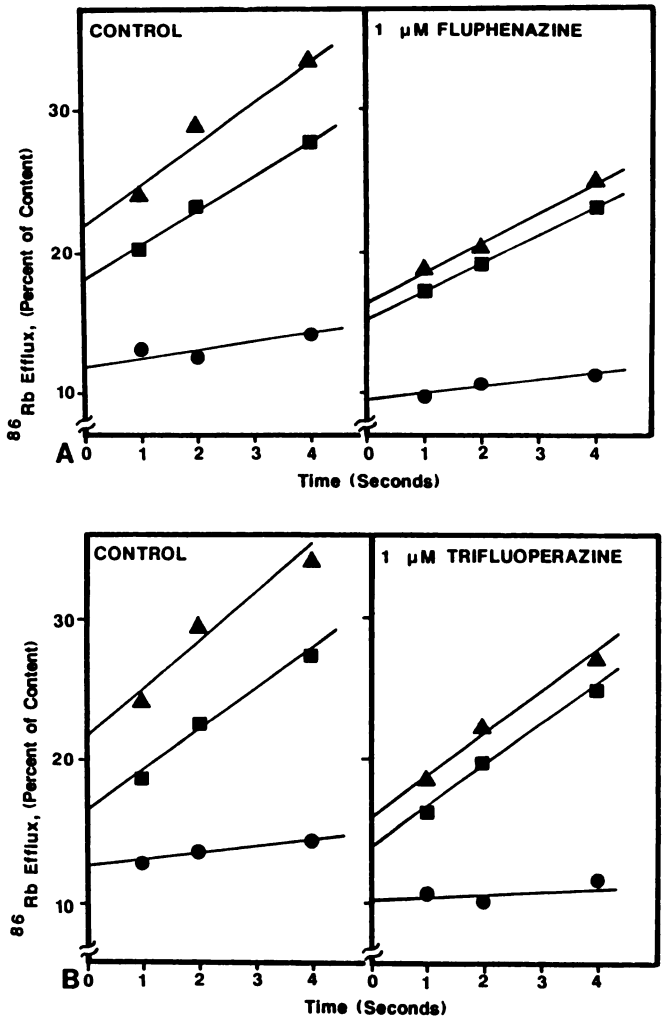

Fig. 2. Effects of 1 μM FLU (A) and 1 μM TFP (B) on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured for 1–4 sec in the absence (control) or presence of drug, as indicated: ●, efflux into 5K; ■, efflux into 100K; ▲, efflux into 100K/Ca. The data in A and B are from two different, representative experiments.

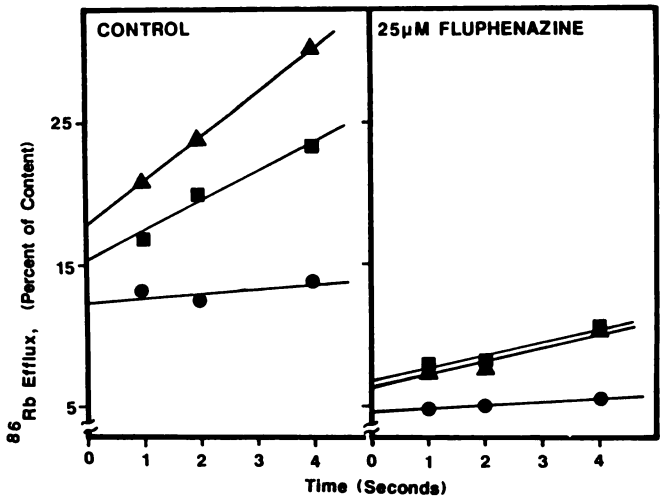

Fig. 3. Effect of 25 μM FLU on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured in the absence or presence of drug, as indicated: ●, efflux into 5K; ■, efflux into 100K; ▲, efflux into 100K/Ca.

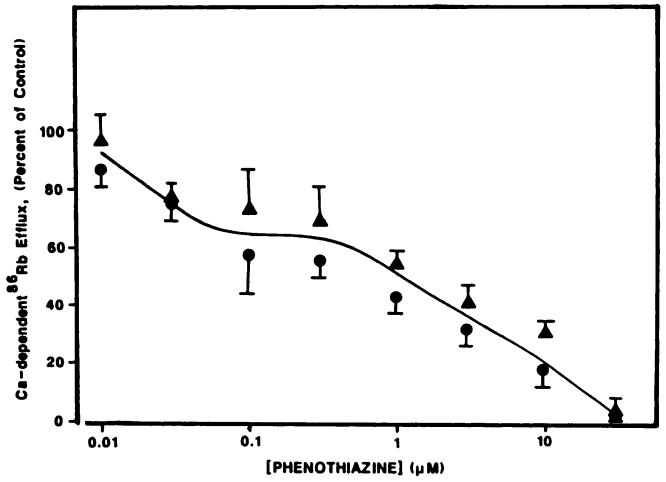

Fig. 4. Dose response curves showing the effects of FLU (▲) and TFP (●) on ^{86}Rb efflux from synaptosomes. The Ca-dependent efflux (difference between efflux into 100K/Ca and 100K) at 2 sec (see Figs. 1–3) is plotted as a function of the phenothiazine concentration. The data are normalized to the Ca-dependent Rb efflux in the absence of drug (= 100%). Each *symbol* represents the mean of data from four different synaptosome preparations; *bars* indicate standard errors.

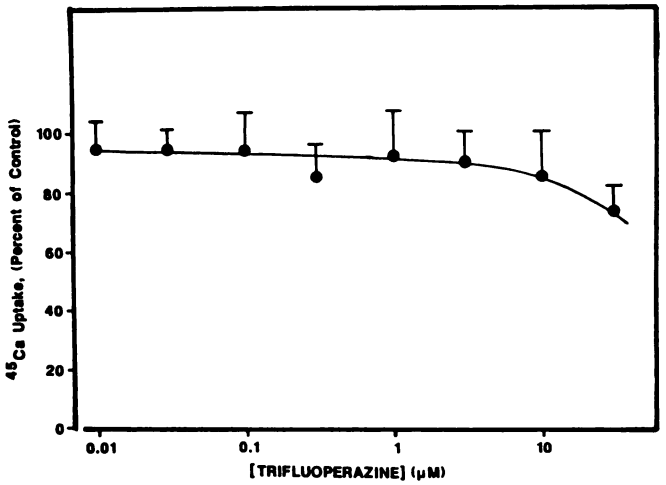

Fig. 5. The effect of TFP on K-stimulated ^{45}Ca uptake by synaptosomes. The data are the differences between the Ca uptakes from 100K/100 μM Ca and 5K/100 μM Ca during a 2-sec incubation (see Materials and Methods) at the indicated concentrations of TFP; the data are normalized to the K-stimulated Ca uptake in the absence of TFP (192 pmol of Ca/mg of protein × 2 sec = 100%). Each *symbol* represents the mean of data from four different synaptosome preparations; *bars* indicate standard errors.

the phenothiazines were less specific for the Ca-activated K channels and inhibited all components of the synaptosome ^{86}Rb efflux, as shown for FLU in Fig. 3.

The dose response curves for the inhibition of Ca-activated K channels by phenothiazines appeared to be biphasic (Fig. 4); this raises the possibility that there may be two populations of channels with different sensitivities to the phenothiazines. Approximately 30–40% of the flux (the "high sensitivity" component) was blocked by a 100 nM concentration of drug; the IC_{50} of this component was 20–30 nM with both FLU and TFP. The remaining 60–70% of the Ca-dependent ^{86}Rb efflux (the "low sensitivity" component) was blocked with an IC_{50} of 1–3 μM TFP or FLU when the synaptosomes were depolarized with 100 mM K. The dose response curves in Fig. 4 were generated by measuring the Ca-dependent ^{86}Rb efflux at a single time

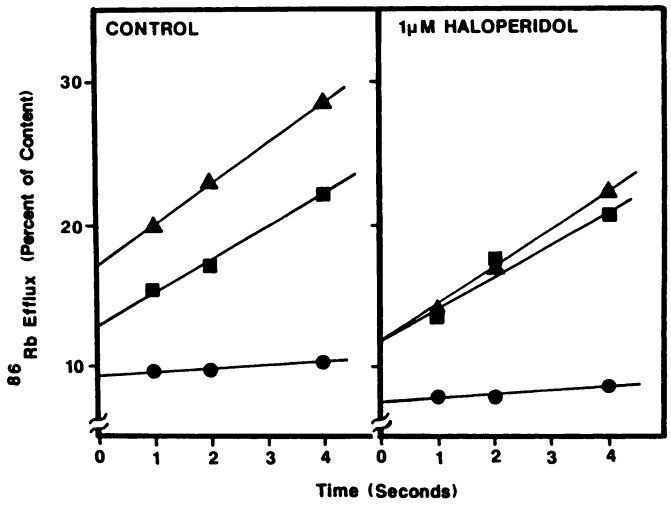

Fig. 6. Effect of 1 μM haloperidol on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured in the absence (control) or presence of drug: ●, efflux into 5K; ■, efflux into 100K; ▲, efflux into 100K/Ca.

point (2 sec). Similar results were obtained at 1 and 5 sec, and when the phenothiazines were added to the ^{86}Rb loading solution for 30 min to prolong the exposure to the drugs (data not shown).

We also tested the effects of PRO, a phenothiazine with little antidopaminergic or antipsychotic activity (Table 2). In contrast to FLU and TFP, PRO had relatively little effect on the Ca-dependent ^{86}Rb efflux: 10 μM PRO inhibited this efflux by only 18 ± 7% (mean ± SE of four experiments).

The gating of Ca-activated K channels normally requires Ca^{2+} influx through voltage-gated Ca channels (22). Therefore, to determine whether inhibition of the Ca-activated K conductance was due to inhibition of Ca conductance, the effects of TFP and FLU on synaptosome K-stimulated ^{45}Ca uptake were directly examined. With up to 10 μM TFP or FLU, a concentration that substantially inhibited Ca-dependent ^{86}Rb efflux (Fig. 4), there was only a minimal effect on K-stimulated ^{45}Ca uptake (data for TFP are shown in Fig. 5). Higher concentrations of TFP and FLU (>30 μM) did inhibit Ca uptake (data not shown) as observed by others (30).

Effects of other antipsychotic agents and anticalmodulin agents on ^{86}Rb efflux from synaptosomes. The sensitivity of Ca-activated K channels to the phenothiazines raised the possibility that the gating of these channels may involve calmodulin. In order to examine this possibility further, several other drugs with greater and lesser anticalmodulin activity were examined. The anticalmodulin properties and the affinities of these compounds for dopamine receptors (measured by displacement of ^{3}H-haloperidol), as reported in the literature, are summarized in Table 2.

Haloperidol is a butyrophenone antipsychotic with antidopaminergic potency comparable to that of the phenothiazines, and with similar efficacy as an anticalmodulin agent (Ref. 31; see Table 2). From the data in Fig. 6 it is apparent that 1 μM haloperidol is also a selective inhibitor of the Ca-activated K channels. However, in contrast to the apparently biphasic dose response curves for the phenothiazines (Fig. 4), the dose response curve for the inhibition of the Ca-dependent Rb efflux by haloperidol was monophasic, with an IC_{50} of 0.5 μM (Fig. 7). Haloperidol, like the phenothiazines, only minimally inhibited

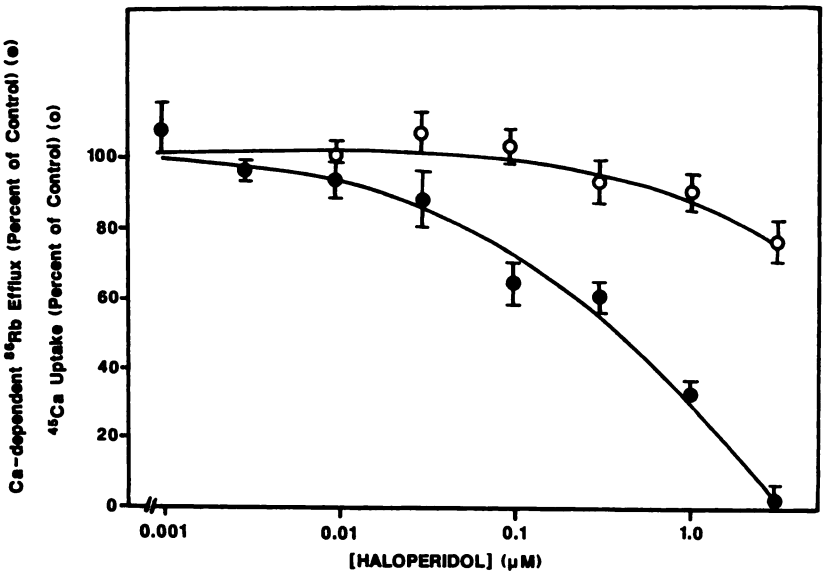

Fig. 7. Dose response curve showing the effects of haloperidol on Ca-dependent ^{86}Rb efflux (●) and K-stimulated ^{45}Ca influx (○) in synaptosomes. The Ca-dependent Rb efflux (difference between efflux into 100K/Ca and 100K) at 2 sec (see Figs. 1–3) is plotted as a function of the drug concentration. The data are normalized to the Ca-dependent Rb efflux in the absence of drug (= 100%). Each *symbol* (●) represents the mean of data from three different synaptosome preparations; *bars* indicate standard errors. The K-stimulated Ca influx data are normalized to the uptake in the absence of drug (= 100%; see Fig. 5 legend). Each symbol (○) indicates the mean of data from four different synaptosome preparations; *bars* indicate standard errors.

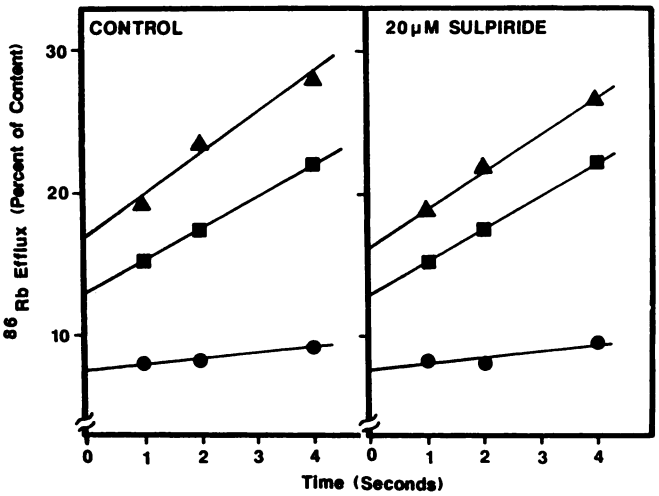

Fig. 8. Effect of 20 μM sulpiride on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured in the absence or presence of drug, as indicated: ●, efflux into 5K; ■, efflux into 100K; ▲, efflux into 100K/Ca.

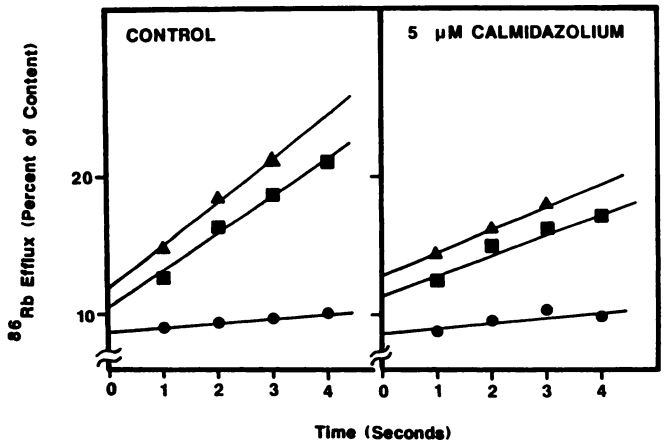

Fig. 9. Effect of 5 μM calmidazolium on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured in the absence or presence of drug, as indicated: ●, efflux into 5K; ■, efflux into 50K; ▲, efflux into 50K/Ca.

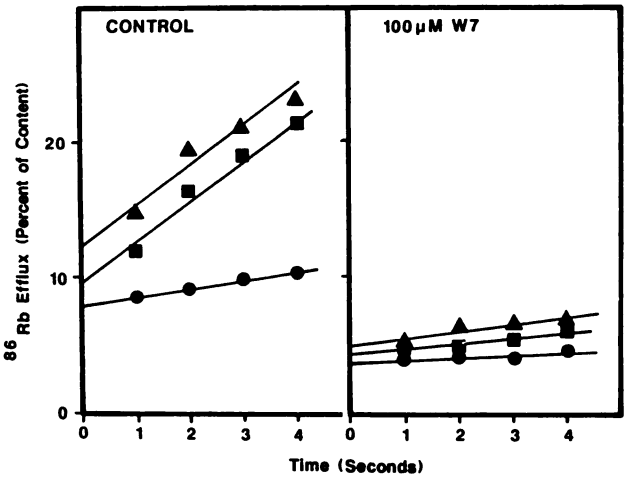

Fig. 10. Effect of 100 μM W7 on the time course of ^{86}Rb efflux from synaptosomes. The efflux was measured in the absence (control) or presence of drug, as indicated: ●, efflux into 5K; ■, efflux into 100K; ▲, efflux into 100K/Ca.

the K-stimulated ^{45}Ca uptake at concentrations up to 1 μM (Fig. 7); thus inhibition of the Ca-dependent Rb efflux could not be explained by block of Ca entry through voltage-gated Ca channels.

Sulpiride is a dopaminergic antagonist with minimal anticalmodulin activity (Ref. 31; see Table 2). Results in Fig. 8 show that a high concentration (20 μM) of sulpiride inhibited the Ca-dependent component of ^{86}Rb efflux by only about 25%. Because the sulpiride-sensitive fraction was only a small proportion of total ^{86}Rb efflux, generation of a dose response curve to sulpiride was not feasible.

Two drugs with more selective anticalmodulin activity were also examined for their ability to block Ca-activated K channels: W7 and calmidazolium (compound R-24571). Low μM concentrations of these drugs (5 μM W7 and 0.5–5 μM calmidazolium) had negligible effects on the synaptosome Ca-activated ^{86}Rb efflux; however, higher concentrations of both agents caused nonselective inhibition of ^{86}Rb efflux. Fig. 9 shows the effect of 5 μM calmidazolium; its most pronounced effect is an inhibition of voltage-gated Rb efflux component S,

with minimal effect on the Ca-activated efflux. A high concentration of W7 (100 μM) nonspecifically inhibited all components of synaptosome ^{86}Rb efflux (Fig. 10).

Discussion

The Phenothiazines and Haloperidol Inhibit Ca-Activated K Channels

In the experiments presented here, several types of drugs with differing anticalmodulin and antidopaminergic activity (Table 2) were examined for their ability to block K channels in CNS nerve endings. The results indicate that TFP, FLU, and haloperidol are potent inhibitors of Ca-activated K channels at concentrations that do not affect Ca entry through voltgate-gated Ca channels. This suggests three possible mechanisms of drug action: 1) these drugs interfere with calmodulin-dependent regulation of Ca-activated K channels; 2) these drugs interfere with dopamine receptor-dependent regulation of Ca-activated K channel function; or 3) these drugs interact directly with the Ca-activated K channel molecules to inhibit conductance.

1. **Gating of synaptosome Ca-activated K channels is not regulated by calmodulin.** Two drugs which are more selective anticalmodulin agents, W7 and calmidazolium (Table 2), had little effect on the Ca-dependent ^{86}Rb efflux. On the basis of the rank order of potency of the anticalmodulin effects of these drugs and the others we tested in this study (Table 2), it appears unlikely that calmodulin mediates the action of Ca on Ca-dependent K channels: there was no correlation between the anticalmodulin potency of these agents and their ability to block Ca-activated K channels. High concentrations of phenothiazines (25 μM) and W7 (100 μM) had a nonselective depressant action on all components of ^{86}Rb efflux.

The observation that anticalmodulin drugs influence ion fluxes is not novel. The effect of anticalmodulin drugs on K conductances has also been examined in human erythrocytes: Lackington and Orrego (32) reported inhibition of K efflux from human erythrocytes by high (10^{-5}–10^{-4} M) concentrations of FLU, chlorpromazine, and W7.

Phenothiazines also inhibit Ca uptake in a number of tissues, including synaptosomes (30). As shown here, however, the inhibition of Ca uptake cannot account for the effect of these drugs on Ca-activated K channels because the direct effect on Ca uptake is observed only at relatively high concentrations of the drugs.

Recently, Dinan *et al.* (33) reported that several neuroleptics block late AHP, which is due to a Ca-activated K conductance, in rat hippocampal pyramidal cells. They found that μM concentrations of haloperidol, chlorpromazine, TFP, and sulpiride inhibited the AHP but did not interfere with Ca spikes. They ruled out an action based on block of dopamine receptors and favored the view that these drugs acted by interfering with calmodulin; however, they did not consider a direct effect of these agents on the Ca-activated K channels.

2. **Gating of synaptosome Ca-activated K channels is not mediated by dopaminergic receptors.** The phenothiazines and haloperidol both inhibited the entire Ca-dependent component of ^{86}Rb efflux, although the concentrations required for half-maximal inhibition were 100- to 500-fold higher than the apparent dissociation constants (K_D) for drug-dopamine receptor binding. In the case of the phenothiazines, the inhibition appeared to be biphasic: about 30–40% of the Ca-dependent ^{86}Rb efflux appeared to be particularly sensitive to the phenothiazines. The IC_{50} values for this "high sensitivity" component (20–30 nM) are closer to the reported K_D values for FLU and TFP binding to dopaminergic receptors (1–5 nM), but still approximately 10-fold higher. Sulpiride, another potent dopamine antagonist, also selectively inhibited the Ca-dependent ^{86}Rb efflux without inhibiting Ca-independent or basal ^{86}Rb efflux. However, a high concentration of sulpiride (10–20 μM) was required to demonstrate partial block (Fig. 8); this is well above its K_D for binding to dopamine receptors (Table 2). Because of these discrepancies between the IC_{50} values for K channel inhibition and the rank order of K_D values for dopamine receptor binding (Table 2), it seems unlikely that the synaptosome Ca-activated K channels are directly associated with a presynaptic dopamine receptor.

There is considerable evidence that the clinical efficacy of the phenothiazines and haloperidol is related to their antidopaminergic activity (29). Our results raise the possibility that some clinical side effects of these drugs may be due, at least in part, to inhibition of Ca-activated K channels; the range of blood concentrations attained during clinical therapy (29) is well within the range which we have shown to inhibit Ca-activated K channels.

3. **Phenothiazines and haloperidol interact directly with the Ca-activated K channel to inhibit conductance.** The findings presented here are consistent with a recent report that several neuroleptic agents inhibit Ca-activated K channels in smooth muscle cells (34). These single channel studies indicate that the inhibitory drugs bind directly to, and block, the open K channel. The dissociation constants for drug binding (1.0 μM for haloperidol and 1.6 μM for TFP) are consistent with the IC_{50} values obtained for inhibition of the Ca-dependent ^{86}Rb efflux in synaptosomes. Additionally, single channel measurements of rat brain Ca-activated K channels incorporated into planar lipid bilayers indicate that these channels are blocked by 0.5–2.0 μM concentrations of the phenothiazines and haloperidol, as well as by higher concentrations of W7, in the absence of exogenous calmodulin.[2] These observations, as well as our own, indicate that these drugs all act directly on Ca-activated K channels.

There is considerable evidence that there may be several classes of Ca-activated K channels in neuronal (4–6) and non-neuronal (35, 36) tissues. Electrophysiological observations on vertebrate neurons indicate that there are at least two different classes of Ca-activated K channels with different kinetic and pharmacological properties. For example, the large conductance, "maxi" Ca-activated K channels, which are blocked by TEA and charybdotoxin (27), rapidly inactivate and appear to participate in action potential repolarization (37, 38). Another Ca-activated K channel, which is insensitive to charybdotoxin and TEA, underlies an AHP lasting many msec; it controls the rate of repetitive firing and accommodation in vertebrate neurons (6, 37, 38). The charybdotoxin, TEA, haloperidol, and phenothiazine sensitivities of the Ca-dependent ^{86}Rb efflux described in this report (see Refs. 22 and 26) suggest that it is mediated by the "maxi" Ca-activated K channel rather than the channel responsible for the AHP. Recently, Reinhart and Levitan (39) reported that there may be three different charybdotoxin-sensitive Ca-activated K channels in rat brain as

[2] B. K. Krueger and D. K. Bartschat, unpublished data.

studied by reconstitution in planar lipid bilayers. In synaptosomes, blockage of Ca-dependent ^{86}Rb efflux by phenothiazines (Fig. 4), but not haloperidol (Fig. 7), appears to reveal more than one component of efflux and may reflect this heterogeneity. Further experiments will be required to resolve this issue.

The results presented in this report indicate that certain phenothiazines and haloperidol have an effect on neuronal tissue in addition to those effects described in the past. These other actions include antagonism of dopaminergic receptors, which may be responsible for the antipsychotic activity of these agents (29), as well as antagonism of muscarinic receptors (40, 41) and antagonism of calmodulin (see Table 2). However, our results indicate that low concentrations of FLU, TFP, and haloperidol selectively block Ca-activated K channels in rat brain presynaptic nerve terminals by a direct action on these channels. This interaction may be useful for biochemical characterization and isolation of these channels.

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