British Journal of Pharmacology (1997) 120, 1114–1118

Inhibition of synaptic transmission and epileptiform activity in central neurones by fluspirilene

Su-Jane Wang, Kwok-Tung Lu & [1]Po-Wu Gean

Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan City, Taiwan 701

1 Recent studies have shown that fluspirilene, a dopamine D_2 receptor antagonist which is a long-acting neuroleptic useful in the maintenance therapy of schizophrenic patients, also displays Ca^{2+} channel blocking activity. In the present study, we have investigated the effect of fluspirilene on synaptic transmission and epileptiform activity induced in slices of hippocampus and amygdala.

2 Fluspirilene reversibly suppressed the field excitatory postsynaptic potential (f-e.p.s.p) in a concentration-dependent manner in the area CAl of the hippocampus without affecting the size and shape of fibre volley. Fluspirilene also inhibited the intracellularly recorded e.p.s.p. in amygdala neurones without affecting the resting membrane potential or neuronal input resistance.

3 Fluspirilene increased the ratio of paired-pulse facilitation suggesting a presynaptic mode of action.

4 Epileptiform activity induced in the disinhibited slices was suppessed by fluspirilene in a concentration-dependent manner. This antiepileptic effect was occluded in slices pretreated with the adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA).

5 It is concluded that fluspirilene-induced synaptic inhibition is probably due to a reduction in presynaptic Ca^{2+} currents. In clinical trials, the low incidence of seizures provoked by fluspirilene might be related to its intrinsic ability to inhibit synaptic transmission and epileptiform activity.

Keywords: Fluspirilene; amygdala; hippocampus; epilepsy; synaptic transmission

Introduction

The class of drugs known as neuroleptics, which are used to treat psychiatric disorders, are antagonists of dopamine D_2 and D_2-like receptors including D_3 and D_4 subtype receptors (Seeman, 1980; Sokoloff *et al*., 1990; Van Tol *et al*., 1991). Many neuroleptic drugs, especially aliphatic phenothiazines, should be used with extreme caution because they can lower the seizure threshold and induce discharge patterns in the EEG that are associated with epileptic seizure disorder (Baldessarini, 1996). In contrast, the occurrence of convulsive phenomenon after administration of piperazine and piperidine phenothiazines is rare (Colasanti, 1990).

Fluspirilene, a member of the diphenylbutylpiperidine phenothiazines, is an effective long-acting neuroleptic particular useful in the maintenance therapy of schizophrenic patients (Hassel, 1985). Interestingly, fluspirilene and other diphenylbutylpiperidines also display a Ca^{2+} channel blocking action (Gould *et al*., 1983; Galizzi *et al*., 1986). Biochemical studies have shown that these drugs prevent the binding of Ca^{2+} channel blockers to cortical membrane vesicles (Gould *et al*., 1983) and to a variety of tissues (Quirion *et al*., 1985; Qar *et al*., 1987; King *et al*., 1989; Kenny *et al*., 1990). Whole cell patch clamp experiments revealed that diphenylbutylpiperidines blocked both low- and high-threshold Ca^{2+} channels in rat pituitary growth hormone (GH) cell lines (Enyeart *et al*., 1990). Recently, Sah & Bean (1994) and Grantham *et al*. (1994) further demonstrated that P-type Ca^{2+} channels in cerebellar Purkinje neurones and N-type Ca^{2+} channels in sympathetic neurones and nerve growth factor-differentiated PC12 cells were inhibited by diphenylbutylpiperidines, and that fluspirilene was the most potent of the drugs tested. In view of the involvement of N- and P-type Ca^{2+} channels in synaptic transmission in the central nervous system (Kamiya *et al*., 1988; Turner *et al*., 1992; Wheeler *et al*., 1994), it is of particular interest to see whether the Ca^{2+} channel blocking activity of fluspirilene is paralleled by its low incidence of provoking seizure. In the present study, we have investigated the effect of fluspirilene on synaptic transmission and the epileptiform activity induced in disinhibited brain slices.

Methods

Brain slice preparation

Brain slices were prepared as described previously (Rainnie *et al*., 1991). Briefly, Male Sprague-Dawley rats (125–150 g) were decapitated and the brains rapidly removed from the skull. Coronal slices nominally 500 μm thick were cut and the appropriate slices were placed in a beaker of artificial cerebrospinal fluid (ACSF). The ACSF was bubbled continuously with 95% O_2-5% CO_2 to maintain the proper pH (7.3–7.5). The composition of the ACSF solution was (in mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11.

The slices were kept at room temperature for at least 1 h before recording. A single slice was then transferred to the recording chamber where it was held submerged between two nylon nets and maintained at 32 ± 1°C. The chamber consisted of a circular well of a low volume (1–1.5 ml) and was constantly perfused at a rate of 3–4 ml min^{-1}.

Extracellular recordings in hippocampal slices

Extracellular recordings of field excitatory postsynaptic potentials (f-e.p.s.ps) and population spikes were obtained from stratum radiatum and stratum pyramidale by use of microelectrodes filled with 3 M NaCl (4–8 MΩ). A bipolar stimulating electrode (SNE-200X, Kopf Instruments, Tujunga, CA, U.S.A.) was placed in the stratum radiatum. The stimulus strength was adjusted to produce the amplitude of f-e.p.s.p. around 0.3–0.5 mV and the amplitude of population spike around 1 mV. The strength of synaptic transmission was quantified by measuring the initial slope of the f-e.p.s.p. and the amplitude of population spikes. To isolate fibre volleys, recordings were made in the presence of 6-cyano-7-ni-

[1] Author for correspondence.

troquinoxaline-2,3-dione (CNQX, 10 μM), D-amino-5-phosphonovalerate (D-APV, 20 μM) and bicuculline (20 μM) to block postsynaptic potentials. Stimulating electrodes were placed closer than usual to the recording electrode to facilitate fibre volley recording.

Intracellular recordings in amygdalar slices

Intracellular recordings were obtained from neurones of the basolateral amygdala nucleus by conventional intracellular recording techniques. Microelectrodes were pulled from microfibre-filled 1.0 mm capillary tubing on a Brown-Flaming electrode puller (Sutter Instruments, Novato, CA, U.S.A.). The electrodes were filled with 4 M potassium acetate with resistance ranging from 60 to 120 MΩ. A bipolar stimulating electrode was placed in the lateral nucleus of the amygdala. For most experiments, stimulus intensities were adjusted just subthreshold for othodromic spike generation.

For data acquisition and analysis, pClamp 6.0 (Axon Instruments) running on PC486 was used. All data are expressed as mean $\pm$ s.e.mean. Statistical analysis was performed by use of Student's t test and a P value less than 0.05 was considered to be statistically significant.

Fluspirilene (Research Biochemicals International, Natick, MA, U.S.A.) was dissolved in a dimethylsulphoxide (DMSO) stock solution and kept frozen until the day of experiment. It was then added to the ACSF and the final concentration adjusted.

Results

Effect of fluspirilene on the synaptic transmission

In order to investigate the effect of fluspirilene on synaptic transmission, recordings of f-e.p.s.p. were performed in the dendritic region of hippocampal CAl area. Fluspirilene reversibly decreased the slope of the f-e.p.s.p. in a concentration-dependent manner (Figure 1a). At concentrations of 10 and 30 μM, fluspirilene suppressed the slope of f-e.p.s.p. by an average of $23.3 \pm 3.7\%$ and $46.9 \pm 5.8\%$ ($n=9$) respectively (Figure 1b). This effect was not due to a decrease in presynaptic excitability because it was not accompanied by a reduction in the amplitude of the fibre volley. To quantify this effect, we isolated the fibre volley by recording the field potentials in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), D-2-amino-5-phosphonovalerate (D-APV, 20 μM), and bicuculline (20 μM) to block postsynaptic potentials and by placing the stimulating electrode closer than usual to the recording electrode. As illustrated in Figure 1c, fluspirilene did not affect the amplitude of the afferent volley ($n=6$).

Intracellular recordings were made from the basolateral nucleus of the amygdala. Afferent stimulation evoked an e.p.s.p. mediated by glutamate acting predominantly on AMPA receptors (Rainnie *et al*., 1991). Figure 2 shows that superfusion of fluspirilene depressed the amplitude of the e.p.s.p. The effect of fluspirilene was slowly reversible when perfusing solution was switched to control ACSF that did not contain the drug. At concentration of 10 μM, fluspirilene depressed the amplitude of e.p.s.p. by $45.4 \pm 4.5\%$ ($n=6$, $P<0.001$). A transient hyperpolarizing current pulse (100 pA, 50 ms) was applied before the evoked responses to monitor the neuronal input resistance. As noted in Figure 2, fluspirilene had no effect on the input resistance. The input resistance was 42.2 ± 3.4 MΩ before and 41.7 ± 2.8 MΩ ($n=6$) after the application of fluspirilene.

Paired-pulse facilitation

Paired-pulse facilitation (PPF) of neurotransmission has been studied extensively and has been attributed to a presynaptic Ca^{2+} accumulation during repetitive stimuli which triggers a proportionally larger amount of transmitter release. It is generally assumed that an increase in the ratio of the second pulse response to the first pulse response (P_2/P_1) indicates a decrease in release probability, because manipulations that depress transmitter release usually increase the magnitude of P_2/P_1 (Manabe *et al*., 1993; Schulz *et al*., 1994). Therefore we compared the magnitude of P_2/P_1 before and after the treatment with fluspirilene. Figure 3a shows synaptic responses to a pair of stimuli with an interstimulus interval of 60 ms. Bath application of fluspirilene (30 μM) increases the magnitude of PPF. The results from 8 experiments are summarized in Figure 3b. P_2/P_1 ratios were 1.21 ± 0.04 in control and 1.46 ± 0.03 in the presence of fluspirilene ($P<0.001$, $n=8$).

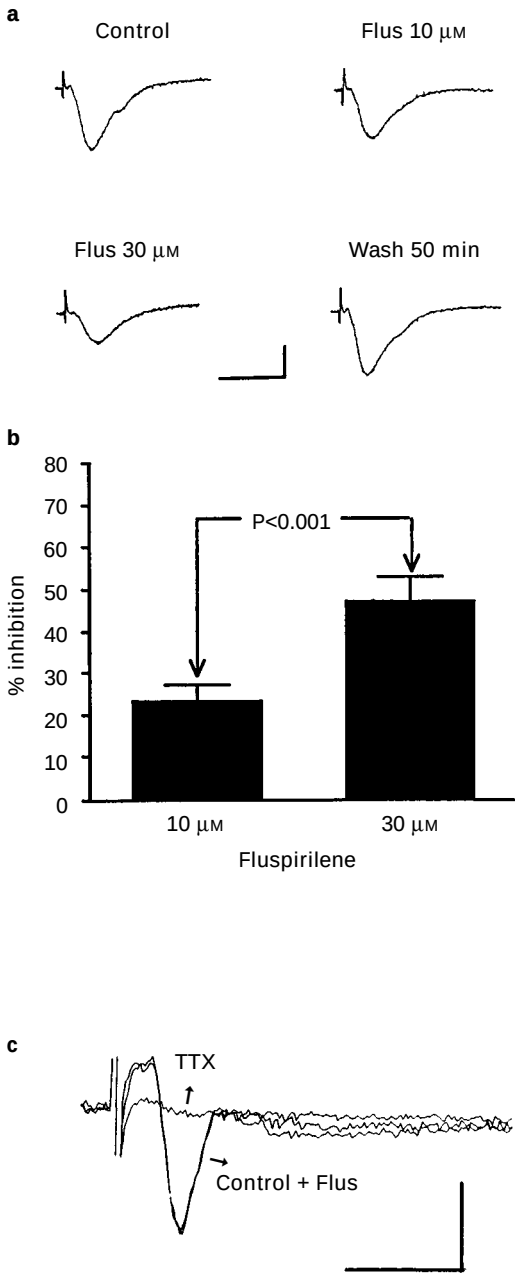

Figure 1 Fluspirilene depresses the f-e.p.s.p. in a concentration-dependent manner. (a) Typical f-e.p.s.ps recorded under control conditions, after exposure to 10 and 30 μM fluspirilene (Flus), and 50 min after washout of the drug. Calibration: 20 ms, 0.2 mV. (b) Concentration-dependent depression of the f-e.p.s.p. by fluspirilene ($n=9$). (c) Fluspirilene had no detectable effect on the isolated fibre volleys. Recordings were made in the presence of CNQX (10 μM), D-APV (20 μM) and bicuculline (20 μM). The fibre volley was completely blocked by tetrodotoxin (TTX, 1 μM). Calibration: 5 ms, 0.2 mV.

Antiepileptic effect of fluspirilene

After blockade of γ-aminobutyric acid$_A$ ($GABA_A$) receptors by bicuculline (20 μM), epileptiform activity manifested as 3–4 additional population spikes could be induced in the hippocampal CAl neurones. The mean amplitudes of the first three population spikes were 2.7 ± 0.3 mV, 1.6 ± 0.2 mV and 1.0 ± 0.2 mV, respectively ($n = 8$). An example of fluspirilene treatment is shown in Figure 4a and pooled data from 8 slices are shown in Figure 4c. When fluspirilene (30 μM) was applied, the amplitude of the first three population spikes was reduced by $45.1 \pm 9.3\%$, $54.1 \pm 8.8\%$ and $61.1 \pm 8.3\%$ respectively.

Similar experiments were performed in the amygdala with intracellular recording techniques. In the presence of bicuculline (20 μM), epileptiform bursts could be recorded in the basolateral amygdala neurones as described previously (Gean & Shinnick-Gallagher, 1987). Figure 5 shows that bath application of fluspirilene (30 μM) shortened the duration of burst discharges. The burst durations were reduced by $34.4 \pm 1.4\%$ and $53.1 \pm 3.6\%$ during the perfusion of 10 μM and 30 μM fluspirilene, respectively (Figure 5b).

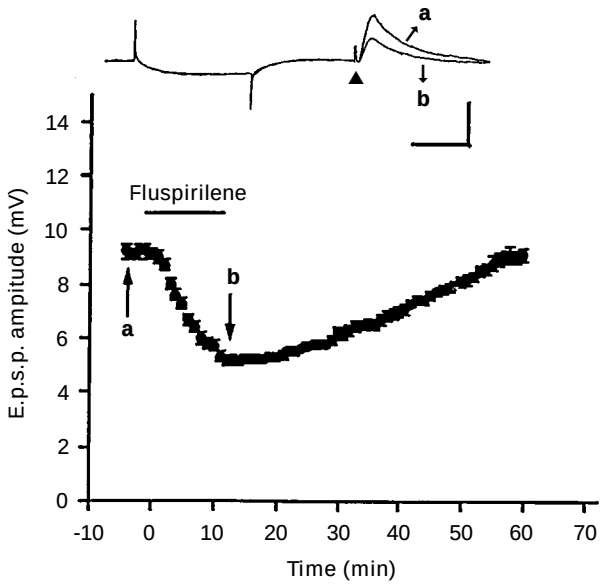

Figure 2 Effect of fluspirilene on the intracellularly recorded e.p.s.p. in the amygdala. The amplitude of the e.p.s.p. was plotted as a function of time. Bar denotes the period of delivery of fluspirilene (10 μM). Inset shows typical records taken before and after the application of fluspirilene. The e.p.s.p. was preceded by a transient hyperpolarizing current pulse (100 pA, 50 msec) passed through the recording electrode to monitor the input resistance. Calibration: 25 msec, 10 mV.

Inhibition of the effect of fluspirilene by N^6-cyclopentyladenosine

Many excitatory synapses in the mammalian brain are depressed by activation of presynaptic adenosine A_1 receptors. Inhibition of Ca^{2+} influx is thought to contribute to changes in synaptic strength (Wu & Saggau, 1994; Dittman & Regehr, 1996). To see whether fluspirilene and the adenosine A_1 receptor agonist, N^6-cyclopentyladenosine (CPA) affected overlapping components of Ca^{2+} influx, we compared the extent of inhibition of epileptiform activity by fluspirilene in the presence and absence of CPA. Epileptiform activity was induced by bicuculline (20 μM). Figure 4b shows that application of CPA (10 nM), depressed the amplitude of population spikes. Subsequent fluspirilene application in the presence of CPA failed to affect the epileptiform activity. The amplitudes of the

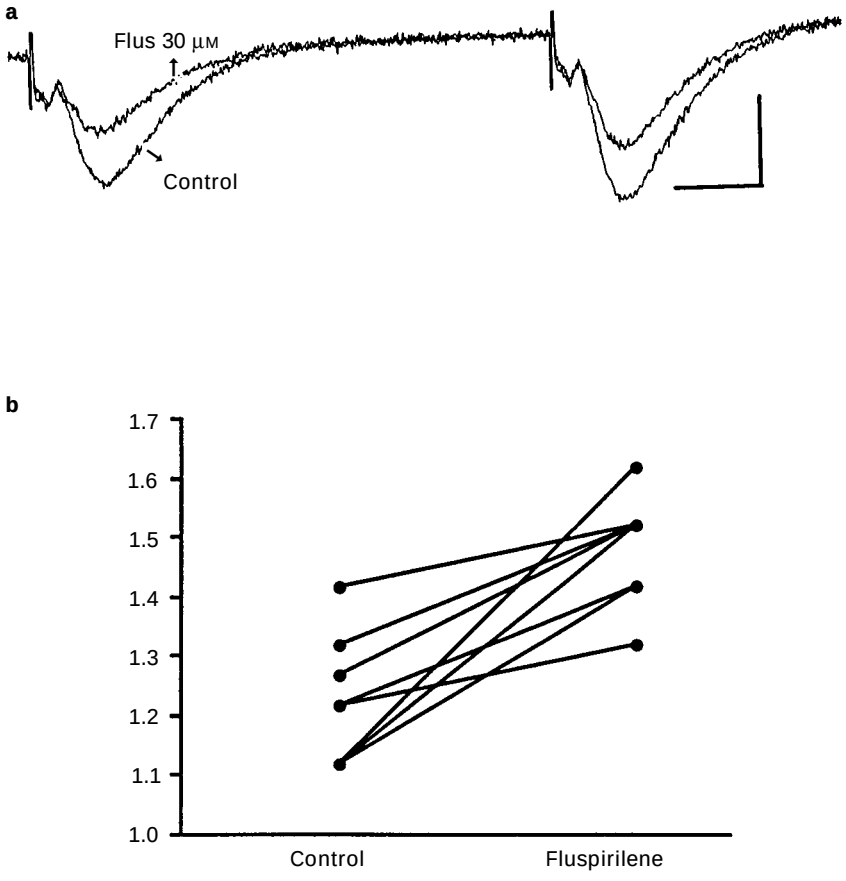

Figure 3 Paired-pulse facilitation is enhanced by fluspirilene. (a) Sample records of f-e.p.s.ps evoked by paired stimuli (60 ms interval) in the control condition and after application of fluspirilene (30 μM). Calibration: 10 ms, 0.2 mV. (b) Data from 8 cells showing effect of fluspirilene on paired-pulse facilitation.

first, second and third population spikes were reduced by 45.1±9.3%, 54.1±8.8% and 61.1±8.3% in control and 5.0±2.6%, 5.6±4.5% and 9.2±4.3% in the presence of 10 nM CPA ($n=8$, $P<0.001$) (Figure 4c).

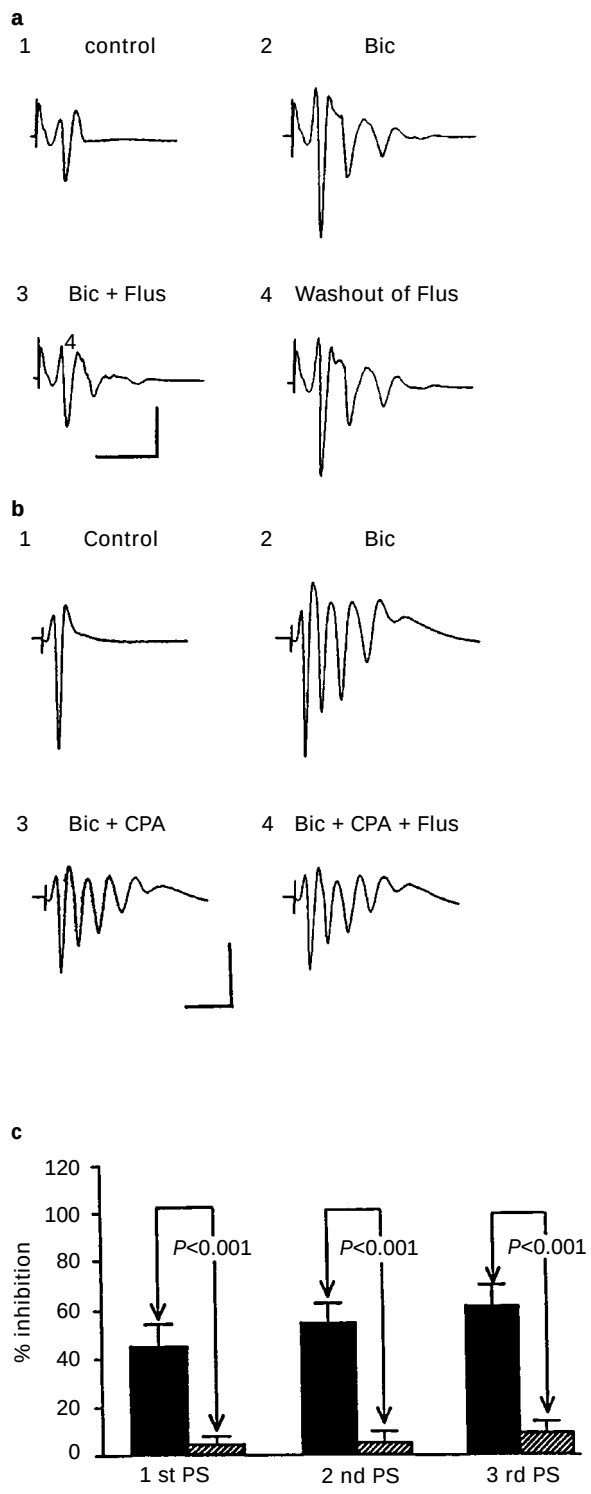

Figure 4 Antiepileptic effect of fluspirilene and the occlusion of the effect of fluspirilene by an adenosine A_1 receptor agonist. (a) Effect of fluspirilene (Flus, 30 μM) on the evoked population spikes in the presence of bicuculline (Bic, 20 μM). Population spikes (PS) were recorded in the CA1 region under control condition (1), generation of epileptiform activity by bicuculline (2) and its inhibition by fluspirilene (3). Calibration: 20 ms, 1 mV. (b) N^6-cyclopentyladenosine (CPA) occluded the effect of fluspirilene. Population spikes were recorded under control condition (1), generation of epileptiform activity by bicuculline (20 μM) (2) and its inhibition by CPA (10 nM) (3) and CPA + fluspirilene (30 μM) (4) Calibration: 20 ms, 1 mV. (c) Histogram of pooled data ($n=8$) showing the inhibitory effect of fluspirilene on the epileptiform activity in the absence (solid columns) and presence (hatched columns) of CPA (10 nM).

Discussion

The present experiments demonstrated that fluspirilene depresses excitatory synaptic transmission in area CA1 of hippocampal slices and basolateral nucleus of amygdalar slices. A number of possible mechanisms for the action of fluspirilene were elucidated in this study. We first examined whether fluspirilene acted presynaptically to reduce transmitter release or postsynaptically to block glutamate receptors. Although we did not exclude the possibility that fluspirilene interacts directly with N-methyl-D-aspartate (NMDA) or non-NMDA receptors, we considered it most likely that fluspirilene acts at the level of presynaptic terminal for the following reasons. Firstly, fluspirilene depresses synaptic transmission with negligible changes in resting membrane potential or input resistance of postsynaptic neurones. Secondly, paired-pulse facilitation, a presynaptic phenomenon, was enhanced during the reduction of f-e.p.s.p.s. Thirdly, the effect of fluspirilene was occluded by an adenosine A_1 receptor agonist.

The mechanism by which fluspirilene reduces transmitter release is unknown. Fluspirilene could act by blocking voltage-

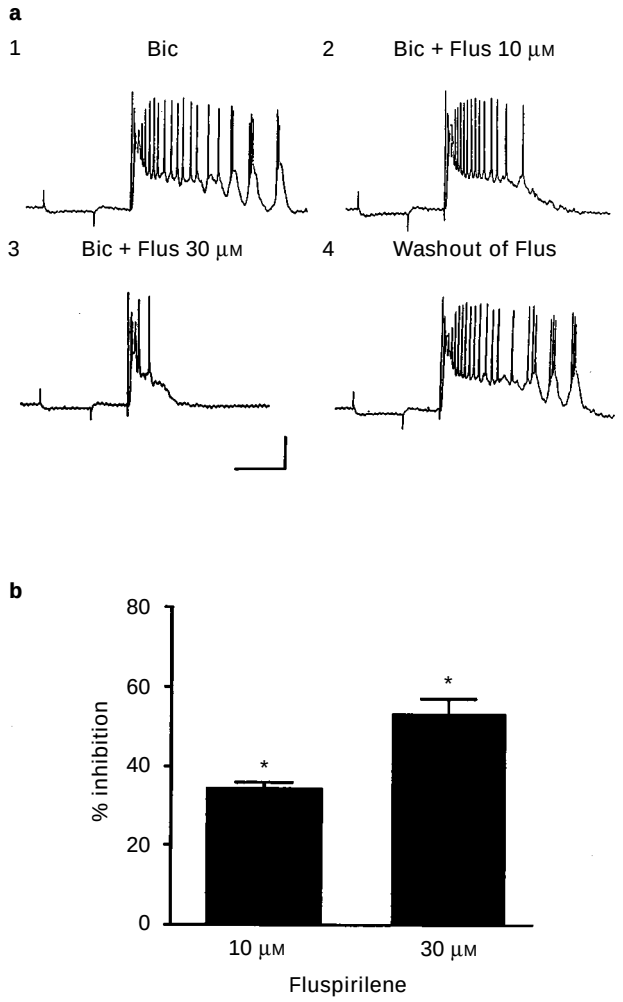

Figure 5 Effect of fluspirilene on the epileptiform activity induced by bicuculline in the amygdala (a1) In the presence of bicuculline (Bic, 20 μM), afferent stimulation evoked a burst response. (a2 and a3) The duration of epileptiform activity was reduced by 10 and 30 μM fluspirilene (Flus). (a4) Response 40 min after washout of fluspirilene. Calibration: 200 ms, 20 mV. (b) Graphic analysis of the effect of fluspirilene on the epileptiform activity. *$P<0.01$ vs control ($n=5$).

dependent Na^+ channels, thus stabilizing the presynaptic neuronal membrane and reducing the release of transmitter. Alternatively, fluspirilene may activate or enhance K^+ conductance resulting in hyperpolarization of the nerve terminal and a reduction in transmitter release. However, fluspirilene had no discernible effect on the shape or size of fibre volley, indicating that changes in presynaptic axonal excitability cannot account for the action of fluspirilene on synaptic transmission (Wheeler *et al*., 1996). It is suggested, therefore, that at least a part of the central action of fluspirilene may be the result of an effect on transmitter release through its antagonism of presynaptic Ca^{2+} influx. These results are consistent with the findings of Sah & Bean (1994) and Grantham *et al*., (1994); who, by using the whole-cell patch clamp technique, showed fluspirilene blocks N- and P-type Ca^{2+} channels.

The EC_{50} values of fluspirilene to suppress the P- and N-type Ca^{2+} currents were 6 μM and 2 μM, respectively (Sah & Bean, 1994), which are comparable to our finding that 10 μM fluspirilene depressed the intracellularly recorded e.p.s.p. by 45.4%. However, these concentrations are considerably higher than corresponding therapeutic doses (2.0 mg per week). Since block of P-type Ca^{2+} channels by fluspirilene was enhanced by depolarized holding potentials and by more frequent depolarizing pulses (Sah & Bean, 1994), it is suggested that in the therapeutic concentration range fluspirilene may not influence normal synaptic transmission but selectively affect synchronized epileptic activity.

Excessive glutamatergic transmission has been implicated in the pathology of epilepsy (Loscher, 1993). Therefore, inhibition of presynaptic Ca^{2+} channels such as N- and P-type Ca^{2+} channels would be expected to reduce excessive transmitter release, thereby preventing spread of neuronal excitation. Indeed, it has been shown that antagonists of the presynaptic Ca^{2+} channels exhibit anticonvulsant activity in *in vitro* (Robichaud *et al*., 1994) and *in vivo* (Gandolfo *et al*., 1989) models of epilepsy. Thus, fluspirilene may have a major advantage in treating psychotic patients who might be at risk from seizures. Furthermore the ablity of fluspirilene to inhibit transmitter release via a presynaptic blockade of N- and P-type Ca^{2+} channels might suggest its potential use as a neuroprotective agent, especially in pathological situations where excessive glutamate release occurs.

This study was supported by the National Science Council of Taiwan (NSC86-2314-B006-002-M10).

References

BALDESSARINI, R.J. (1996). Drugs and treatment of psychiatric disorders: psychosis and anxiety. In *Pharmacological Basis of Therapeutics*. ed. Hardman, P.B. & Limbird, L.E. pp. 399–430. New York: The McGraw-Hill Companies.

COLASANTI, B.K. (1990). Antipsychotic drugs. In *Modern Pharmacology*. ed. Craig, C.R. & Stitzel, R.E. pp. 461–472. Boston: Little, Brown and Company.

DITTMAN, J.S. & REGEHR, W.G. (1996). Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. *J. Neurosci.*, **16,** 1623–1633.

ENYEART, J.J., BIAGI, B.A., DAY, R.N., SHEU, S.S. & MAURER, R.A. (1990). Blockade of low and high threshold calcium channels by diphenylbutylpiperidine antipsychotics linked to inhibition of prolactin gene expression. *J. Biol. Chem.*, **265,** 16373–16379.

GALIZZI, J.-P., FOSSET, M., ROMEY, G., LADURON, P. & LAZDUNSKI, M. (1986). Neuroleptics of the diphenylbutylpiperidine series are potent calcium channel inhibitors. *Proc. Natl. Acad. Sci. U.S.A.*, **83,** 7513–7517.

GANDOLFO, G., GOTTESMANN, C., BIDARD, J.-N. & LADZUNSKI, M. (1989). Ca^{++} channel blockers prevent seizures induced by a class of K^+ channel blockers. *Eur. J. Pharmacol.*, **160,** 173–177.

GEAN, P.W. & SHINNICK-GALLAGHER, P. (1987). Picrotoxin induced epileptiform activity in amygdaloid neurons. *Neurosci. Lett.*, **73,** 149–154.

GOULD, R.J., MURPHY, K.M.M., REYNOLDS, I.J. & SNYDER, S.H. (1983). Antischizophrenic drugs of the diphenylbutylpiperidine type act as calcium channel antagonists. *Proc. Natl. Acad. Sci. U.S.A.*, **80,** 5122–5125.

GRANTHAM, C.J., MAIN, M.J. & CANNELL, M.B. (1994). Fluspirilene block of N-type calcium current in NGF-differentiated PC12 cells. *Br. J. Pharmacol.*, **111,** 483–488.

HASSEL, P. (1985). Experimental comparison of low doses of 1.5 mg fluspirilene and bromazepam in out-patients with psychovegetive disturbances. *Pharmacopsychiatry*, **18,** 297–302.

KAMIYA, H., SAWADA, S. & YAMAMOTO, C. (1988). Synthetic ω-conotoxin blocks synaptic transmission in the hippocampus in vitro. *Neurosci. Lett.*, **91,** 84–88.

KENNY, B.A., FRASER, S., KILPATRICK, A.T. & SPEDDING, M. (1990). Selective antagonism of calcium channel activators by fluspirilene. *Br. J. Pharmacol.*, **100,** 211–216.

KING, V.F., GARCIA, M.L., SHEVELL, J.L., SLAUGHTER, R.S. & KACZOROWISKI, G.J. (1989). Substituted diphenylbutylpiperidines bind to a uniquely high affinity site on the L-type calcium channel. *J. Biol. Chem.*, **264,** 5633–5641.

LOSCHER, W. (1993). Basic aspects of epilepsy. *Curr. Opinion Neurol. Neurosurg.*, **6,** 223–232.

MANABE, T., WYLLIE, D.J.I. & NICOLL, R.A. (1993). Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSP variance in the CAl region of the hippocampus. *J. Neurophysiol.*, **70,** 1451–1459.

QAR, J., GALIZZI, J.P., FOSSET, M. & LAZDUNSKI, M. (1987). Receptors for diphenylbutylpiperidine neuroleptics in brain, cardiac and smooth muscle membranes. Relationship with receptors for 1,4-dihydropyridines and phenylalkylamines and with calcium channel blockade. *Eur. J. Pharmacol.*, **141,** 261–268.

QUIRION, R., LAFAILLE, F. & NAIR, N.P.V. (1985). Comparative potencies of calcium channel antagonists and antischizophrenic drugs on central and peripheral calcium channel binding sites. *J. Pharm. Pharmacol.*, **37,** 437–440.

RAINNIE, D.G., ASPRODINI, E.K. & SHINNICK-GALLAGHER, P. (1991). Excitatory transmission in the basolateral amygdala. *J. Neurophysiol.*, **66,** 986–998.

ROBICHAUD, L.J., WURSTER, S. & BOXER, P.A. (1994). The voltage-sensitive Ca^{++} channel (VSCC) antagonists ω-Aga-IVA and ω-CTX-MVIIC inhibit spontaneous epileptiform discharges in the rat cortical wedge. *Brain Res.* **643,** 352–356.

SAH, D.W., & BEAN, B.P. (1994). Inhibition of P-type and N-type calcium channels by dopamine receptor antagonists. *Mol. Pharmacol.*, **45,** 84–92.

SCHULZ, P.E., COOK, E.P. & JOHNSTON, D. (1994). Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. *J. Neurosci.*, **14,** 5325–5337.

SEEMAN, P. (1980). Brain dopamine receptors. *Pharmacol. Rev.*, **31,** 229–313.

SOKOLOFF, P., GIROS, B., MARTRES, M.-P., BOUTHENET, M.-L. & SCHWARTZ, J.-C. (1990). Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics. *Nature*, **347,** 146–151.

TURNER, T.J., ADAMS, M.E. & DUNLAP, K. (1992). Ca^{++} channels coupled to glutamate release identified by ω-Aga-IVA. *Science*, **258,** 310–313.

VAN TOL, H.J., BUNZOW, J.R., GUAN, H.-C., SUNAHARA, R.K., SEEMAN, P., NIZNIK, H.B. & CIVELLI, O. (1991). Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. *Nature*, **350,** 610–614.

WHEELER, D.B., RANDALL, A. & TSIEN, R.W. (1994). Roles of N-type and Q-type Ca^{++} channels in supporting hippocampal synaptic transmission. *Science* **264,** 107–111.

WHEELER, D.B., RANDALL, A. & TSIEN, R.W. (1996). Changes in action potential duration alter reliance of excitatory synaptic transmission on multiple types of Ca^{++} channels in rat hippocampus. *J. Neurosci.*, **16,** 2226–2237.

WU, L.G. & SAGGAU, P. (1994). Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CAl of hippocampus. *Neuron*, **12,** 1139–1148.

(Received September 23, 1996
Revised December 3, 1996
Accepted December 6, 1996)