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Comparison of anaesthetic and non-anaesthetic effects on depolarization-evoked glutamate and GABA release from mouse cerebrocortical slices

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1 Investigation with substances that are similar in structure, but different in anaesthetic properties, may lead to further understanding of the mechanisms of general anaesthesia.

2 We have studied the effects of two cyclobutane derivatives, the anaesthetic, 1-chloro-1,2,2-trifluorocyclobutane (F3), and the non-anaesthetic, 1,2-dichlorohexafluorocyclobutane (F6), on K^+-evoked glutamate and γ-aminobutyric acid (GABA) release from isolated, superfused, cerebrocortical slices from mice, by use of h.p.l.c. with fluorescence detection for quantitative analysis.

3 At clinically relevant concentrations, the anaesthetic, F3, inhibited 40 mM K^+-evoked glutamate and GABA release by 72% and 47%, respectively, whereas the structurally similar non-anaesthetic, F6, suppressed evoked glutamate release by 70% but had no significant effects on evoked GABA release. A second exposure to 40 mM KCl after a $\sim$30 min washout of F3 or F6 showed recovery of K^+-evoked release, suggesting that F3 and F6 did not cause any non-specific or irreversible changes in the brain slices.

4 Our findings suggest that suppression of excitatory neurotransmitter release may not be directly relevant to the primary action of general anaesthetics. A mechanism involving inhibitory postsynaptic action is implicated, in which a moderate suppression of depolarization-evoked GABA release by the anaesthetic may be consistent with the enhancement of postsynaptic GABAergic activities.

Keywords: Neurotransmitter; γ-aminobutyric acid (GABA); glutamate; general anaesthetic; 1-chloro-1,2,2-trifluorocyclobutane (F3); non-anaesthetic; 1,2-dichlorohexafluorocyclobutane (F6); brain slice; high performance liquid chromatography (h.p.l.c.)

Introduction

Mechanisms of action of general anaesthetics are yet to be elucidated. At the cellular level, there seems to be a consensus that general anaesthetics exert their action by modulating synaptic transmission, rather than affecting action potential propagation, in the central nervous system (Griffiths & Norman, 1993; Pocock & Richards, 1993; Franks & Lieb, 1994; Richards, 1995). Glutamate and γ-aminobutyric acid (GABA) are, respectively, the major excitatory and inhibitory neurotransmitters in the mammalian brain (Dagani & D'Angelo, 1992; Mohler, 1992; Griffiths & Norman, 1993). Volatile anaesthetics have been shown to decrease reversibly the depolarization- or ischaemia-evoked release of glutamate in isolated cerebral synaptosomes (Schlame & Hemmings, 1995; Miao *et al*., 1995), neuronal cell cultures (Zhu & Baker, 1996), cortical and hippocampal brain slices (Larsen *et al*., 1994; Bickler *et al*., 1995), and the parietal cortex and dorsal hippocampus *in situ* (Patel *et al*., 1995). The effects of general anaesthetics on GABA release are more complex; whereas some anaesthetics can enhance GABA release, many suppress it (for review, see Pocock & Richards, 1993; Richards, 1995). Moreover, several commonly used clinical volatile anaesthetics have been found to have no effects on GABA release (Lecharny *et al*., 1995; Mantz *et al*., 1995), yet these agents can potentiate GABAergic inhibitory postsynaptic currents (Jones *et al*., 1992; Lin *et al*., 1992; Mihic *et al*., 1994; Zimmerman *et al*., 1994; Mihic & Harris, 1996).

Many volatile anaesthetics obey the Meyer-Overton rule that the potency of these agents correlates with their solubility in olive oil. Recently, compounds that are similar in structure and oil solubility but different in anaesthetic properties have become available (Taheri *et al*., 1993; Koblin *et al*., 1994; Liu *et al*., 1994a,b). Comparison of the action of these compounds allows for identification of the properties that are essential for the production of general anaesthesia (Raines & Miller, 1994; Tang *et al*., 1997). Among many structurally similar pairs of anaesthetics and non-anaesthetics are two fluorinated cyclobutanes: 1-chloro-1,2,2-trifluorocyclobutane (F3) and 1,2-dichlorohexafluorocyclobutane (F6). Although both are predicted to be anaesthetics by the Meyer-Overton rule (Koblin *et al*., 1994), only F3 has been shown to be an anaesthetic (Kendig *et al*., 1994; Mihic *et al*., 1994; Dibly-Mayfield *et al*., 1996). In fact, F6 is an anti-anaesthetic so that instead of producing anaesthesia by itself or reducing the requirement for a conventional anaesthetic, it actually increases the requirement of a conventional anaesthetic to produce general anaesthesia (Kendig *et al*., 1994).

In the present study, we have investigated the effect of F3 and F6 on the K^+ depolarization-evoked release of glutamate and GABA from mouse cerebrocortical slices. We showed that the anaesthetic, F3, and the non-anaesthetic, F6, modulate glutamate and GABA release differently. Our results support the notion that the principal action of general anaesthetics is the potentiation of postsynaptic inhibitory activity (Franks & Lieb, 1994; 1996), rather than the suppression of transmitter release.

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Methods

Brain slice preparation

Experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Twenty-one male adult mice (F_2 hybrid of C57BL/6J × Strain 129), weighing 20.2 ± 0.5 g, had unlimited access to food and water. For each experiment, a mouse was decapitated, and its brain was rapidly excised and flushed with a cold, oxygenated (95% O_2 and 5% CO_2), artificial cerebrospinal fluid (oxy-ACSF), which normally contains (in mM): NaCl 123.9, $NaHCO_3$ 15.8, glucose 10.0, $MgSO_4$ 1.1, KH_2PO_4 1.2, $CaCl_2$ 1.2 and KCl 5.0. Four, 350 μm-thick cortical slices, two dorsal and two lateral, were manually cut from the brain, by the method described previously (Espanol *et al*., 1994). The time required for the procedure was less than a minute. After cutting, slices were immediately placed into a thermostatic chamber (37 ± 0.5°C) and constantly superfused with a non-circulating flow of oxy-ACSF at a rate of $(1.00 \pm 0.01) \times 10^{-3}$ l min^{-1}. The total volume of ACSF in the chamber was maintained at a constant of 0.5×10^{-3} l.

Superfusion apparatus

In order to control precisely the oxygenation of ACSF and the concentration of KCl, F3, or F6 in the superfusion chamber, a programmable syringe pump (KD Scientific, Inc., Boston, MA) was used. One of the 60 ml syringes was the primary one and contained fully oxygenated ACSF. Five secondary syringes contained naturally oxygenated ACSF with precalculated concentrations of KCl, F3, F6, KCl plus F3, or KCl plus F6, respectively. Syringes to be used in each given experiment were all mounted onto the same syringe pump and infused simultaneously. The contents of the primary and one of the secondary syringes were mixed at a 'Y' junction immediately before entering the superfusion chamber. A miniature flow valve was used to switch between the secondary syringes, thereby varying the concentrations of KCl, F3 or F6 in the chamber. With this set-up, nearly constant oxygenation in the chamber (~420 mmHg) was obtained throughout the experiments, without the potential complications caused by the administration of the volatile agents. The ACSF effluent from the chamber was collected continuously with an automatic fraction collector (Gilson Microfraction Collector, Model 203, Middleton, WI) and immediately processed for high performance liquid chromatography (h.p.l.c.) measurements (see below). The precalculated concentrations of F3 and F6 in the chamber were also confirmed by gas chromatography (Perkin-Elmer 8500; Poropak P resin; 150°C), by use of the method described previously (Xu *et al*., 1996; Tang *et al*., 1997).

Experimental protocol

The amount of glutamate and GABA release from the slices was measured in the ACSF effluent, by use of h.p.l.c. with fluorescence detection (Palmer *et al*., 1993). Twenty-one mice were placed randomly into the following four experimental groups: control ($n = 6$), F3 ($n = 5$), F6 ($n = 6$), and Ca^{2+}-free ($n = 4$) groups. For the Ca^{2+}-free group, 1.2 mM $CaCl_2$ in the ACSF was replaced with 1.2 mM $MgCl_2$ throughout the experiment. The experimental procedures consisted of 4 time segments: (1) stabilization (40 min), (2) basal release measurements (2.5–8 min) and pre-equilibration with F3 or F6, if applicable (5 min), (3) K^+-evoked depolarization (1.5 min, 40 mM KCl), along with F3 or F6 if applicable; and (4) recovery period (16 min).

During the 40 min stabilization period, slices were superfused with a 50:50 mixture of oxy-ACSF and naturally oxygenated ACSF, but no release measurement was made. To determine the basal release, four ACSF samples were collected over an 8 min period. The beginning of the first sample collection was arbitrarily designated as time 0 (t = 0). For the F3 or F6 group, F3 or F6 was introduced at t = 3 min to pre-equilibrate with slices for 5 min before K^+-evoked depolarization. At t = 8 min, the KCl concentration in the ACSF was switched to 40 mM for 1.5 min, followed by a 16 min washout with normal oxy-ACSF (5 mM KCl). The continuous outflow of ACSF was collected at a sampling rate of 2 samples per min for 8 min, resulting in 16 samples to define the profile of the K^+-evoked amino acid release. Three additional samples were collected at a slower rate toward the end of the recovery period.

To provide an estimate of the time profile of KCl concentration in the chamber after the 1.5 min concentration elevation, the exact same experimental protocol was repeated with a known concentration of dye in place of KCl. The effluent from the chamber was sampled at a rate of 4 samples per min. The dye concentrations in the samples were measured with a Spectrophotometer (Model DU 650; Beckman Instruments Inc., Fullerton, CA).

In addition, to confirm that exposure to F3 or F6 did not cause irreversible changes in the brain slices, an additional 11 mice were placed randomly into the control ($n = 4$), F3 ($n = 4$), and F6 ($n = 3$) groups. The brain slices were subjected to two consecutive 1.5 min KCl exposures, with the second exposure starting at 30.5 min after the onset of F3 or F6 washout. Samples for h.p.l.c. analysis were collected at 6 min intervals before, during and after KCl exposures, and the ratios of the first to second total evoked release were calculated and compared. To estimate the extent of washout at the time for the second KCl exposure, the tissue concentration of F3 was measured in parallel experiments by ^{19}F nuclear magnetic resonance (n.m.r.) spectroscopy. Brain slices were quickly dried on a piece of filter paper and then homogenized in 0.8 ml heptane. A conventional one-pulse n.m.r. method was used to quantify the ^{19}F n.m.r. spectral intensities.

Amino acid quantification by h.p.l.c. with fluorescence detection

Immediately after being collected by the automatic microfractional collector, an aliquot of 250 μl of ACSF was taken from each ACSF sample, vigorously mixed with 500 μl of acidified methanol (8.4 ml of 0.1 M HCl per 100 ml of methanol) to precipitate the released proteins from the brain slices and centrifuged at 16,000 *g* for 20 min (Francis & Lowe, 1993). The supernatant layers were then filtered through a 0.2 μm nylon syringe filter and quantified by use of h.p.l.c. with fluorescence detection. A reverse-phase column (150 × 4.6 mm) and a guard column (15 × 4.6 mm) packed with octadecylsilane particles (5 μm, Dynamax Microsorb; Rainin Instrument Co., Woburn, MA) were used to achieve separation. Glutamate and GABA were detected as fluorescent derivatives after pre-column derivatization with *o*-phthaldialdehyde, by a fluorescence detector (Model 121, Gilson Inc., Middleton, WI). Aliquots (40 μl) of the filtered supernatant layer were mixed with 40 μl of a mixture of *o*-phthaldialdehyde and 2-mercaptoethanol (Sigma Chemical Company, St. Louis, MO) and reacted for 1 min before being injected onto the column by an automatic sampler (Model 717, Waters Chromatography, Milford, MA). A curvilinear gradient was delivered at a rate of

1×10^{-3} l min^{-1} that was 23% of h.p.l.c.-grade methanol in an h.p.l.c. grade phosphate buffer (50 mM H_3PO_4 + 50 μM EDTA, pH = 5.7) for 6 min, changed in a convex profile from 23% to 44% methanol from 6 to 13 min, remained isocratic (44% methanol) until 26.5 min, and finished with a 5 min elution with 80% methanol. Peaks for glutamate and GABA were identified by comparing the retention times with those of the standards. Peak areas were integrated and calibrated against the standard curves for quantification, by use of Unipoint software (Gilson Inc., Middleton, WI).

Protein content determination

At the end of the recovery period, slices were removed from the chamber and dissolved in a 0.2% solution of Triton X-100. The amount of solubilized protein was measured according to the method of Bradford (1976), by u.v. spectrophotometry.

Calculation of amino acid concentration and statistical analysis

The amount of amino acids in the ACSF samples collected outside the superfusion chamber depends on the weight of the slices, the rate at which the amino acids are released, and the rate of the flow that brings the amino acid out of the chamber. To normalize the effects of the ACSF flow rate and the protein content in the slices, the release of amino acids was calculated as the product of the h.p.l.c.-measured amino acid concentrations and the flow rates, divided by the total protein content in the brain slices, and expressed in units of pmol min^{-1} mg^{-1} protein. Data were first analysed by use of repeated measures ANOVA with the SPSS statistical package (SPSS Inc., Chicago, IL) to determine the effects of KCl-evoked depolarization and cyclobutanes on glutamate and GABA release over time. If the null hypothesis was rejected at $P = 0.05$ for the degrees of freedom (d.f.) of the experiments, the significance of changes in K^+-evoked glutamate and GABA release due to F3 and F6 was then analysed by a one-way ANOVA procedure with Student-Neuman-Keuls and Duncan *post-hoc* multiple comparisons. For the Ca^{2+}-free group, the difference between basal and K^+-evoked release was compared with zero, by use of the two-tailed Student's *t* test. All data are presented as mean ± s.e.mean.

Results

Figure 1 shows representative h.p.l.c. chromatograms of a basal sample and a sample at t = 9 min (1 min after being switched to high KCl concentration), along with a chromatogram of an authentic standard containing 0.113 and 0.165 μM of glutamate and GABA, respectively. Peaks with 9.7 and 21.3 min retention time correspond to glutamate and GABA, respectively.

Figure 2a and b depict changes in glutamate and GABA release, respectively, as a function of time for the control, F3, and F6 groups. The cross-hatched horizontal bar, applicable only to the latter two groups, indicates the period when F3 or F6 was administered in the superfusing ACSF. The equilibrium concentrations of F3 and F6 in the aqueous phase in the chamber, measured by GC, were 1.60 ± 0.11 and 0.117 ± 0.007 mM (mean ± s.e.mean, $n = 3$), respectively. The solid horizontal bar indicates the period (1.5 min) when ACSF was switched to the higher KCl concentration. The probable time profile of elevated KCl in the effluent from the chamber, as estimated in parallel experiments with a combined use of dye and spectrophotometry, is plotted in Figure 2c. The slight

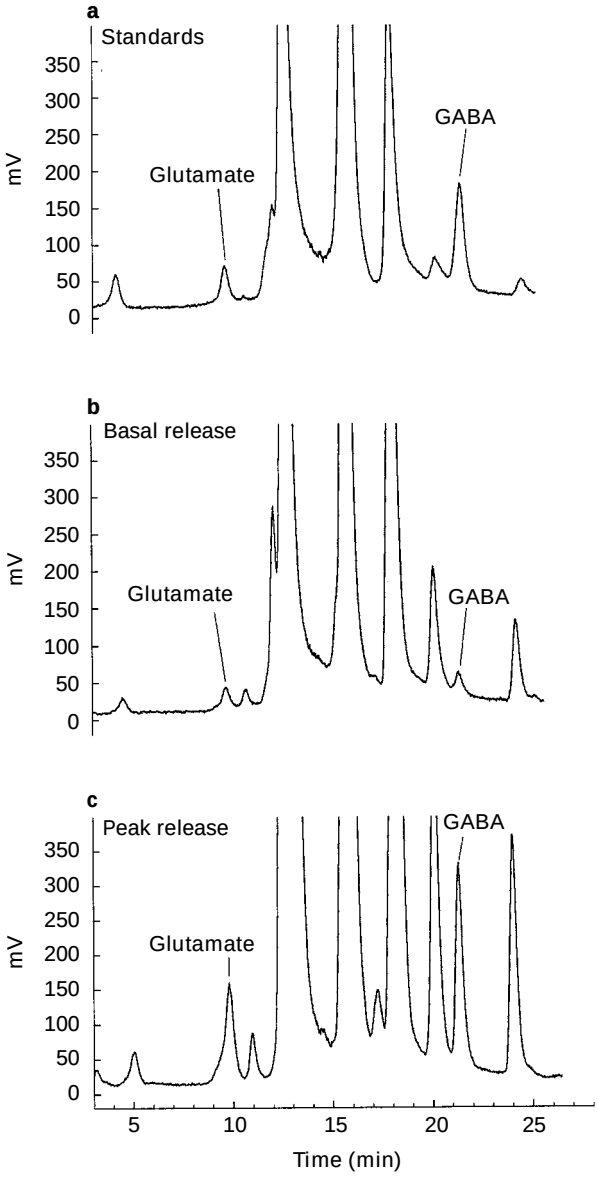

Figure 1 Representative h.p.l.c. chromatograms of (a) 40 μl of an authentic amino acid mixture containing 0.113 μM glutamate and 0.165 μM GABA, and of (b) the basal and (c) the 40 mM KCl-evoked amino acid release. The retention times for authentic glutamate and GABA were 9.7 ± 0.4 min and 21.3 ± 0.5 min, respectively. Quantities of amino acids were determined by use of calibration curves obtained from measuring peak areas of multiple standard samples with different concentrations of amino acids.

delay in the profile relative to the switching reflects the time taken for ACSF to flow through the entire superfusion system.

As shown in Figure 2a and b, the amounts of glutamate and GABA release under the basal condition were 76.5 ± 3.1 and 41.1 ± 2.8 pmol min^{-1} mg^{-1} protein, respectively. Superfusion with 40 mM KCl caused immediate increases in both glutamate and GABA release in the control group (up to 359.7 ± 27.7 and 173.0 ± 21.0 pmol min^{-1} mg^{-1} protein, respectively), which returned to the basal level after KCl concentration was lowered to the normal value (5 mM). The repeated measures ANOVA for the effects of 'treatments' by time between the control, F3 and F6 groups showed significant changes in glutamate ($F = 3.43$, d.f. = 32, $P < 0.001$) and GABA ($F = 1.85$, d.f. = 32, $P = 0.007$) release. When Ca^{2+} in the ACSF was replaced by an equal amount of Mg^{2+} (the Ca^{2+}-free group), repeated measures ANOVA showed no significant changes over time in the release

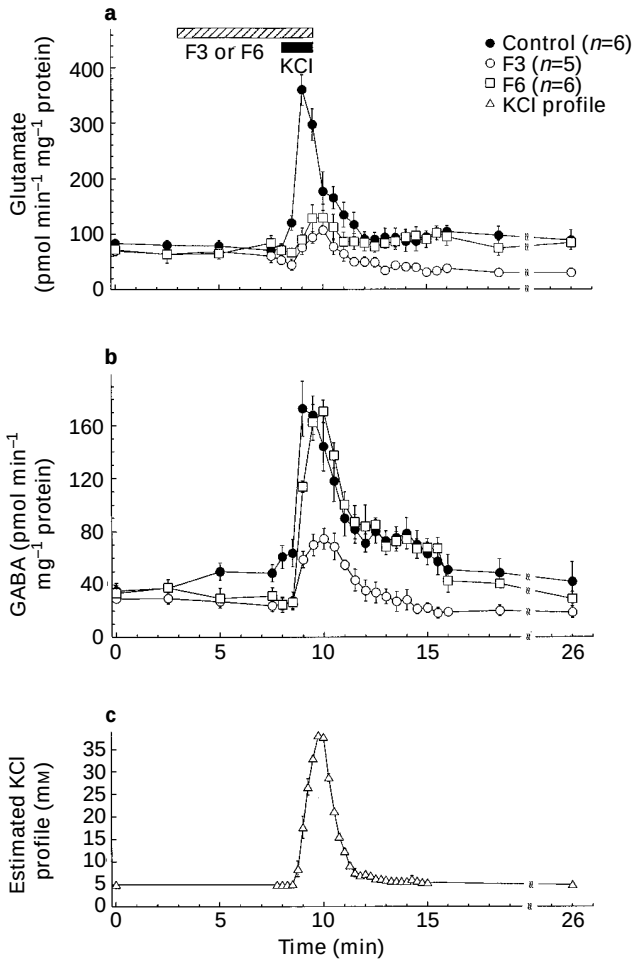

Figure 2 Glutamate (a) and GABA (b) release from isolated, superfused, mouse cerebrocortical slices in response to 1.5 min of 40 mM KCl. The likely time-course of K^+ elevation, as shown in (c), was estimated in parallel experiments by use of spectrophotometry with dye. The cross-hatched horizontal bar indicates the duration of F3 or F6 administration for the corresponding group. Repeated measures ANOVA for the effects of treatments (control, F3 and F6) by time showed significant differences in glutamate ($F=3.43$, d.f. = 32, $P<0.001$) and GABA ($F=1.85$, d.f. = 32, $P=0.007$) release.

of glutamate (49.1 ± 2.4 pmol min^{-1} mg^{-1} protein, $F=1.31$, d.f. = 21, $P=0.226$) or GABA (35.9 ± 1.7 pmol min^{-1} mg^{-1} protein, $F=1.14$, d.f. = 21, $P=0.346$), despite the 1.5 min elevation in KCl concentration in ACSF.

After the corresponding basal levels had been subtracted, glutamate and GABA release values were integrated as a function of time from t = 8 to 16.5 min. The integrals, corresponding to the total K^+-evoked release, are depicted in Figure 3 for the control, F3, F6, and Ca^{2+}-free groups. One-way ANOVA with Student-Neuman-Keuls and Duncan multiple comparisons showed that the anaesthetic, F3, significantly suppressed the K^+-evoked glutamate (by 72%, $P<0.01$) and GABA (by 47%, $P<0.05$) release, whereas the structurally similar non-anaesthetic, F6, significantly suppressed glutamate release (by 70%, $P<0.01$) but not that of GABA. Two-tailed Student's *t* test showed that for the Ca^{2+}-free group, the K^+-evoked release (i.e., release exceeding the basal level) was not significantly different from zero (glutamate $=3.3\pm5.9$ pmol mg^{-1} protein, $P=0.62$; and GABA $=0.14\pm0.07$ pmol mg^{-1} protein, $P=0.20$), suggesting that K^+-evoked release in the other three groups was Ca^{2+}-dependent.

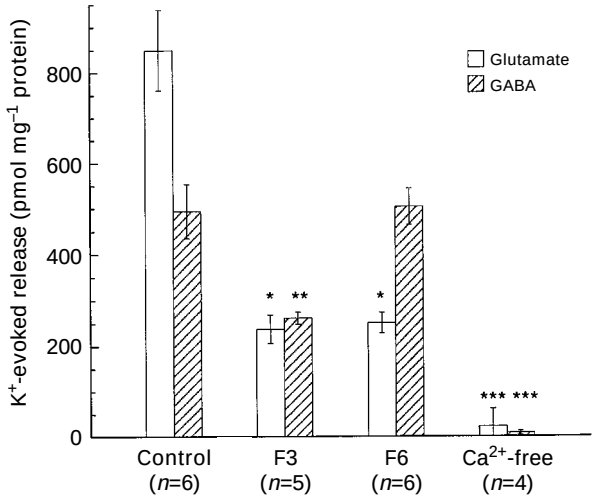

Figure 3 Integrated glutamate and GABA release from isolated, superfused, mouse cerebrocortical slices in response to 40 mM KCl. Compared with the control group ($n=6$), F3 ($n=5$) significantly suppressed 40 mM K^+-evoked glutamate (by 72%, $P>0.01$) and GABA (by 47%, $P<0.05$) release, whereas F6 ($n=6$) significantly suppressed glutamate release (by 70%, $P<0.01$) but not that of GABA. No significant K^+-evoked release was found in the Ca^{2+}-free group. *Significantly different from the corresponding control group; **significantly different from both the corresponding control group and the F6 group; ***significantly different from the control, F3, and F6 groups.

The ratios of the first to second total K^+-evoked release during two consecutive KCl exposures, the first with F3 or F6 and the second after ~30 min washout of F3 or F6, are depicted in Figure 4 for the control, F3 and F6 groups. Clearly, the two KCl exposures caused similar release under the control condition (i.e., without F3 or F6 during the first KCl exposure). Note that after ~30 min of F3 and F6 washout, the slices resumed the normal K^+-evoked release; the percentage of suppression in the first release relative to the second after washout was nearly the same as that shown in Figure 3.

Discussion

At a clinically relevant concentration, the volatile anaesthetic, F3, significantly suppressed the Ca^{2+}-dependent, K^+-evoked glutamate and GABA release from the superfused mouse cerebrocortical slices. The non-anaesthetic, F6, suppressed glutamate release but not that of GABA. The actions of F3 and F6 seemed to be specific, because after a ~30 min washout of F3 or F6, the K^+-evoked glutamate and GABA release recovered to normal levels. *In vivo* magnetic resonance spectroscopy studies (Xu *et al*., 1995) have shown that a ~30 min washout is sufficient to eliminate only the relatively immobile components of hydrophobic volatile anaesthetics from brain tissue. The non-specific, relatively mobile components persist for much longer. Indeed, in the present study with heptane extraction and ^{19}F magnetic resonance spectroscopy (data not shown), we found that at the time coincident with the second KCl exposure, there was still about 15–20% F3 in the tissue. However, this residual amount of F3 seemed to have no effects on glutamate and GABA release. Moreover, the differing actions of F6 on glutamate *versus* GABA release also suggest that some degree of functional selectivity of F6 is operational.

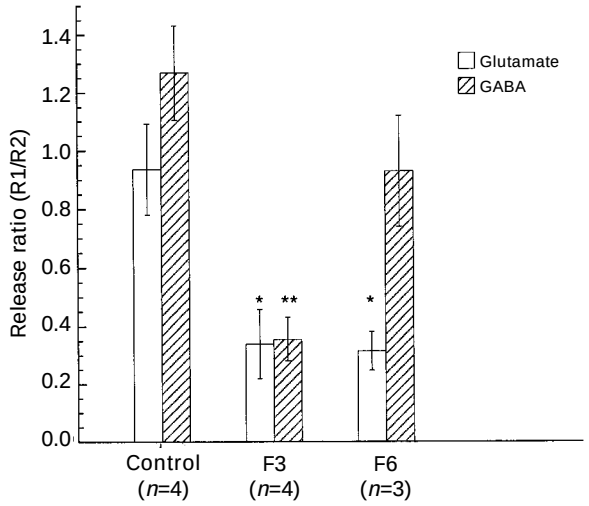

Figure 4 Ratios of the first to second glutamate and GABA release from mouse, isolated, superfused cerebrocortical slices in response to two consecutive 40 mM KCl exposures, with the second one given after ~30 min of F3 or F6 wash-out. Compared with the corresponding release after wash-out, F3 ($n=4$) suppressed the K^+-evoked glutamate and GABA release by 66% and 64%, respectively, whereas F6 ($n=3$) suppressed glutamate release (by 68%) but not that of GABA. *Significantly different from the corresponding control group, $P<0.05$; **significantly different from both the corresponding control group and the F6 group, $P<0.05$.

Many general anaesthetics have been found to depress excitatory transmission at concentrations comparable to that found in the brain during surgical anaesthesia (Bickler *et al*., 1995; Miao *et al*., 1995; Schlame & Hemmings, 1995; MacIver *et al*., 1996a). Our results with F3 on glutamate release is therefore in agreement with these investigations. The effects of anaesthetics on inhibitory synaptic transmission are more complicated, as studies with different agents or in different brain regions have indicated that general anaesthetics can either suppress (Minchin, 1981; Kendall & Minchin, 1982; Osborne *et al*., 1990; Lecharny *et al*., 1995), have no effects on (Mantz *et al*., 1995; MacIver *et al*., 1996b), or enhance (Collins, 1980; 1981; Potashner & Lake, 1981) GABA release. Although such agent- or location-dependent variation on GABA release has been taken as an indication of the complexity associated with the effect of anaesthetics on inhibitory transmission in the central nervous system (Pocock & Richards, 1993), it should be pointed out that a depressant action of general anaesthetics on GABA release, as shown in this study, is not an unexpected finding. If the action of a given anaesthetic is directed at the cellular sites that underlie the release of both the excitatory and inhibitory neurotransmitters (Richards, 1995), then the effects of such an anaesthetic on the release of both transmitters should be expected to be similar.

Anaesthetic modulation of synaptic transmission, whatever the transmitter, can result from manipulation of one or all of the following: transmitter release, transmitter uptake and postsynaptic activities. It has been shown that neither glutamate nor GABA uptake is affected by a variety of general anaesthetics (Jessell & Richards, 1977; Minchin, 1981; Kendall & Minchin, 1982; Nicol *et al*., 1994; 1995). Our method, like many others (for review, see Richards, 1995), is unable to differentiate changes in release from changes in uptake. However, because the amino acids are measured in an ACSF that rapidly flows by the slices and transports amino acids and other released substances away from the slices, it seems rather unlikely that the observed reductions in glutamate and GABA in the effluent after administration of F3 or F6 result from an enhancement of uptake. This view is also supported by the fact that increases in release can be detected in the control group immediately upon exposure to high KCl (Figure 2). Moreover, it has been found that the initial rate of uptake of radiolabelled glutamate into cortical and cerebellar synaptosomes is less than 65 pmol min^{-1} mg^{-1} protein (Nicol *et al*., 1995) and that preincubation for 5–15 min is often required for GABA and glutamate uptake measurements (Mantz *et al*., 1995; Saransaari & Oja, 1995). Given the extent of the decreases shown in Figure 2, our results with F3 and F6 are thus more consistent with a suppression of glutamate and GABA release.

Reduction in extracellular glutamate, mostly due to suppression of release rather than enhancement of uptake, has been proposed as one of the principal mechanisms for general anaesthesia (Larsen *et al*., 1994; Langmoen *et al*., 1995; Schlame & Hemmings, 1995; MacIver *et al*., 1996a,b). However, our results with F6 suggest that the situation is far more complicated, for despite its strong suppressant effects on glutamate release, the non-anaesthetic F6 produces no general anaesthesia. It has been pointed out (Koblin *et al*., 1994; Raines & Miller, 1994) that an action shared by both anaesthetics and non-anaesthetics is unlikely to be relevant to the mechanism of anaesthesia. In contrast, the differing actions of F3 and F6 on GABA release found in this study may infer that one target of anaesthetics is at sites of inhibitory synaptic transmission. In this regard, numerous studies have shown that general anaesthetics can enhance GABAergic synaptic transmission by increasing postsynaptic activities (Jones *et al*., 1992; Lin *et al*., 1992; Mihic *et al*., 1994; Zimmerman *et al*., 1994). Moreover, it has been shown (Lin *et al*., 1992) that such an increase is less profound at higher extracellular GABA concentrations. In fact, the anaesthetic potentiation of GABA-gated Cl^- currents is reduced exponentially, and in some cases reversed, as extracellular GABA concentration increases (Lin *et al*., 1992). Thus, a moderate suppression of K^+-evoked, presynaptic GABA release by F3 may be consistent with its overall enhancement effects on GABAergic action. If depolarization-evoked presynaptic GABA release under normal conditions does not lead to anaesthesia, then a moderate reduction of this release by an anaesthetic may work synergistically with the anaesthetic effects on inhibitory postsynaptic GABAergic currents. This possibility is supported by our findings with F6, because in contrast to F3, F6 had a slight tendency to increase GABA release when compared to the control. Although this tendency was not statistically significant in the present study, we have found in preliminary measurements with 15 min of 40 mM K^+-evoked depolarization that F6 can increase GABA release by as much as 13%, when compared to the control (data not shown). Such an increase, if proven to be physiologically relevant, might explain the anti-anaesthetic property of F6 (Kendig *et al*., 1994) since an increased GABA release by F6 could reduce the potentiation of a conventional anaesthetic on inhibitory postsynaptic currents.

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