Biochemical Pharmacology, Vol. 51, pp. 1613–1621, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00103-7

Effects of Propofol and Thiopentone on Potassium- and Carbachol-Evoked [^{3}H]Noradrenaline Release and Increased [Ca^{2+}]$_i$ From SH-SY5Y Human Neuroblastoma Cells

David G. Lambert, Jonathon M. Willets, Robert Atcheson, Christine Frost, Darren Smart, David J. Rowbotham and Graham Smith*

University Department of Anaesthesia, Leicester Royal Infirmary, Leicester. LE1 5WW. U.K.

ABSTRACT. We have examined the effects of two intravenous anaesthetic induction agents, propofol and thiopentone, on K^+ and carbachol evoked [^{3}H]noradrenaline release from a human neuroblastoma cell line, SH-SY5Y. In this model, we have previously demonstrated that K^+ evoked [^{3}H]noradrenaline release was dependent on Ca^{2+} entry and carbachol evoked release was extracellular Ca^{2+}-independent. Propofol inhibited K^+ (100 mM)-evoked (IC_{50} of 42 ± 11 μM), but not carbachol (1 mM)-evoked, [^{3}H]noradrenaline release. Thiopentone inhibited both K^+- and carbachol-evoked release with IC_{50} values of 116 ± 15 μM and 169 ± 39 μM, respectively. These inhibitory effects were not due to changes in the release dynamics, as assessed using perfused cells. Furthermore, thiopentone inhibition of carbachol-evoked release was not due to muscarinic receptor antagonism. Both propofol and thiopentone caused noncompetitive inhibition of K^+-stimulated Ca^{2+} influx, with IC_{50} values of 127 ± 7 μM and 121 ± 10 μM, respectively. These effects were not due to interaction with $GABA_A$ receptors, but suggest that both compounds block voltage-sensitive Ca^{2+} channels. Thiopentone, but not propofol, inhibited carbachol-stimulated increased intracellular Ca^{2+} concentrations in the presence and absence of extracellular Ca^{2+}. However, thiopentone had no effect on carbachol-stimulated inositol (1,4,5)-triphosphate formation, suggesting that thiopentone may directly inhibit Ca^{2+} release from intracellular stores. BIOCHEM PHARMACOL 51;12:1613–1621, 1996.

KEY WORDS. anaesthetics; propofol; thiopentone; Ca^{2+} channels; depolarisation; muscarinic receptors; noradrenaline release; SH-SY5Y neuroblastoma cells

The cellular mechanism(s) of action of intravenous anaesthetic induction agents, such as propofol (2,6-diisopropylphenol) and thiopentone (a barbiturate), is likely to be multifactorial and, clearly, requires further investigation [1]. However, the synapse represents a potential target site where these agents may interfere with the process of neuronal stimulus-secretion coupling [1, 2]. In addition, the positive correlation between membrane-buffer partition coefficient and the 50% decrease in cortical evoked response in urethane anaesthetised animals, suggests a membrane site of action [3, 4], although nonmembrane sites should also be considered. Although there is a good deal of evidence supporting a general reduction of neurotransmission with barbiturates, including excitatory amino acids [5], GABA [6] and acetylcholine [7], there are very few studies describing the effects of propofol and, for both classes of agents, the mechanism(s) remain to be determined. However, it has been proposed that interaction with $GABA_A$ receptors may be a common mechanism for many anaesthetic agents [4, 8].

Cultured SH-SY5Y human neuroblastoma cells represent a useful human neuronal preparation capable of the uptake and release of noradrenaline [9–15]. We have recently demonstrated in these cells that [^{3}H]noradrenaline release evoked by K^+ depolarization or *via* muscarinic receptor occupation is differentially regulated by extracellular Ca^{2+} [16]. For K^+, the release is entirely extracellular Ca^{2+}-dependent (i.e. Ca^{2+} influx through voltage sensitive channels) and for carbachol the release was extracellular Ca^{2+}-independent, probably utilising Ca^{2+} released from intracellular stores [16]. These findings have been confirmed and substantially extended by others [17]. We have utilised the properties of the release process to probe potential mechanisms of action of propofol and thiopentone. Specifically, if either agent inhibits K^+-evoked release, then the likely target site for this agent is a membrane Ca^{2+} channel. Conversely, if either agent inhibits carbachol-evoked release, an

* Corresponding author. Tel. 0116 2585291 ext. 5474; FAX 0116 2854487.

† *Abbreviations:* [Ca^{2+}]$_i$, intracellular Ca^{2+} concentration; Ins(1,4,5)P_3, inositol 1,4,5-triphosphate; [^{3}H]NMS, 1-[N-methyl-^{3}H]scopolamine methyl chloride; Fura2-AM, Fura-2-acetoxymethylester.

Received 25 September 1995; accepted 8 February 1996.

interaction with intracellular Ca^{2+} release is likely. Direct measurements of intracellular Ca^{2+} concentrations are also reported.

MATERIALS AND METHODS

Cell Culture

Undifferentiated SH-SY5Y human neuroblastoma cells (a gift from Dr. J. Biedler, Memorial Sloane-Kettering Institute for Cancer Research, Rye, NY, U.S.A.) were maintained in minimum essential medium supplemented with 10% foetal calf serum, 2 mM L-glutamine, 100 μg/mL streptomycin, 100 IU/mL penicillin, and 2.5 μg/mL fungizone. Stock cultures (passages 75–95), maintained at 37°C in 5% CO_2/air were passaged weekly and refed twice weekly.

Propofol and Thiopentone Stocks

Pure Propofol (Lot: 71013/91A), a gift from Zeneca (Aklerley Edge, U.K.), was dissolved in DMSO at a concentration of 100 mM and storedat 4°C. Immediately prior to use, propofol was diluted to 0.3 mM in Krebs/HEPES buffer pH 7.4 [14] giving a maximum DMSO concentration of 0.3%, which was included in control tubes; further serial dilutions were made. Thiopentone (a gift from Rhone-Poulenc Rorer, Essex, U.K.) was dissolved in 0.1 M NaOH at 100 mM. This stock was kept at 4°C and used within 3 days. Dilution of this stock to 1 mM in Krebs/HEPES buffer pH 7.4 did not appreciably change the buffer pH. If the pH change exceeded 0.1 units, 0.1 M HCl was titrated to correct this.

Measurement of [^{3}H]noradrenaline Release

STATIC CULTURES. Release of [^{3}H]noradrenaline from statically incubated SH-SY5Y cells was performed using monolayers of cells in 12-well multitrays seeded at 1×10^5 cells/well and cultured (7–8 days) as described previously [11, 15]. Cells were incubated for 60 min with ~50 nM [^{3}H]noradrenaline (36 Ci/mmol, Amersham, U.K.) in Krebs/HEPES buffer pH 7.4. The monolayers were then postincubated for 15 min with 0.5 mL of fresh buffer. This postincubation was repeated twice more. Propofol (3 to 300 μM), thiopentone (33 μM to 1 mM), or DMSO (0.003 to 3%) were added acutely (3 min) or to the final 15-min wash prior to carbachol and K^+ challenge, as described under Results. [^{3}H]noradrenaline release was evoked using a 3 min challenge with either 1 mM carbachol or 100 mM K^+ (with Na^+ adjustment) in 0.5 mL volumes. After a brief centrifugation to remove any cell debris, aliquots (0.25 mL) were counted for radioactivity. [^{3}H]noradrenaline remaining in the monolayer was extracted with 1 mL of perchloric acid (0.4 M) for 30 min. Aliquots (0.25 mL) were counted using HiSafe 3 as a scintillant. Release is expressed as a percentage of total content prior to challenge [11, 15, 16]. In all cases where K^+ depolarization was undertaken, the concentration of K^+ refers to added K^+, in addition to the 5.9 mM present in the normal Krebs/HEPES buffer.

PERFUSED CELLS. SH-SY5Y cells were harvested by brief exposure to HEPES buffered saline pH 7.4 containing 0.02% EDTA. The cells were centrifuged and resuspended in Krebs/HEPES (3 mL) buffer containing ~50 nM [^{3}H]noradrenaline for 60 min. The loaded cells were centrifuged and resuspended in 2 mL of fresh buffer; this was repeated once more. The suspension of cells was then sucked into the perfusion chamber, which consisted of a modified 2-mL syringe barrel containing glass wool at the needle end and a flow diffuser at the plunger end, as described previously [16]. Once loaded, the chamber was reversed and buffer perfused (0.5 mL/min) for a further 15 min. Cells were challenged with 1 mM carbachol or 100 mM K^+ (with Na^+ adjustment) for 3 min. For K^+ stimulation, cells were exposed to two square wave pulses (S_1 and S_2) for 3 min separated by 15 min, during which time propofol (100 μM) or thiopentone (333 μM) were added. In view of the possibility of receptor-mediated desensitization, a single carbachol challenge was used. During these experiments, thiopentone was added to the cells loaded in the chamber for 15 min prior to the addition of carbachol. To control for differing cell densities and, hence, [^{3}H]noradrenaline loading, all data are expressed relative to the first fraction collected [16].

Muscarinic Receptor Binding

The binding of thiopentone to muscarinic receptors on whole SH-SY5Y cells was assessed by the displacement of [^{3}H]NMS,† as described previously [18]. Whole cells were incubated in 1 mL volumes of Krebs/HEPES buffer pH 7.4 for 60 min at 37°C in the presence of a fixed concentration of NMS (~0.2 nM) and increasing concentrations of thiopentone. Bound and free ligand were separated by rapid vacuum filtration onto Whatman GF/B filters using a Brandel cell harvester. Nonspecific binding was determined in the presence of 10 μM atropine.

Measurement of Intracellular Calcium

$[Ca^{2+}]_i$ was measured as described previously [19]. Briefly, confluent SH-SY5Y cells were harvested with the addition of HEPES (10 mM)/EDTA (0.02%), NaCl (0.9%), pH 7.4. After centrifugation, cells were resuspended and washed in Krebs/HEPES buffer, pH 7.4. The cells were then loaded with Fura2-AM (3 μM), for 30 min at 37°C, followed by 20-min postincubation at room temperature to allow complete ester hydrolysis. Finally, intracellular Ca^{2+} was measured fluorometrically, in a Perkin-Elmer LS50B fluorimeter with excitation wavelengths set at 340 and 380 nm, and emission set at 510 nm. $[Ca^{2+}]_i$ was calculated from the 340/380 ratio, according to Grynkiewicz *et al.* [20] where R_{max} and R_{min} were determined using Triton X 100 (0.1% v/v) and EGTA (4.5 mM pH > 8.0). R_{max} and R_{min} values, mean (confidence intervals) were 6.25 (5.79, 6.71) and 0.66 (0.64, 0.68). For potassium experiments, the buffer Na^+ concentration was adjusted accordingly.

Propofol (1 to 300 μM) and thiopentone (3 μM to 1 mM) were either preincubated for 15 min prior to K^+ (20 to 100 mM) or carbachol (1 μM to 1 mM) challenge or added during the sustained phase of increased $[Ca^{2+}]_i$. Appropriate concentrations of DMSO and NaOH were added to all controls and the order of control determinations was varied. To exclude possible interactions of propofol and thiopentone with $GABA_A$ receptors, Fura2-loaded cells were preincubated with GABA (100 μM) prior to depolarization with K^+ (80 mM).

Measurement of Inositol 1,4,5 Triphosphate

$Ins(1,4,5)P_3$ was measured as described by Challis *et al.*, [21]. Briefly, whole-cell suspensions were preincubated for 15 min in Krebs/HEPES buffer pH 7.4, at 37°C, in the presence of either thiopentone (100 or 500 μM) or the equivalent concentration of the vehicle, NaOH, then challenged with carbachol (100 μM) for a maximum of 5 min. In all cases, the reaction was terminated by the addition of ice-cold trichloro acetic acid (0.3 mL of 1 M stock). $Ins(1,4,5)P_3$ was extracted with Freon/octylamine (1:1 v/v) and neutralised with 25 mM $NaHCO_3$. $Ins(1,4,5)P_3$ was assayed using a bovine adrenocortical binding protein and $[^3H]Ins(1,4,5)P_3$ (41 Ci/mmol; Amersham, U.K.) at 4°C. Authentic $Ins(1,4,5)P_3$ (0.036 to 12 pmol; Siemat, U.K.) in buffer taken through an identical extraction process, was used as a standard. Nonspecific binding was defined in the presence of excess $Ins(1,4,5)P_3$ (0.3 nmol). Bound $[^3H]Ins(1,4,5)P_3$ was separated by rapid vacuum filtration [21, 22].

Statistical Analysis

Data are expressed as mean ± SEM or 95% C.I. of at least 4 independent determinations. Data are analysed using Student's paired and unpaired *t*-test and ANOVA as appropriate and considered significant when $P < 0.05$. IC_{50} values were estimated by nonlinear regression analysis, using GraphPad (Version 2.0). Perfusion data were analysed by measuring the area under the normalized release curves. For K^+-stimulated preparations, the S_2/S_1 ratio was calculated. In binding experiments, IC_{50} was corrected for the competing mass of NMS to yield affinity (K_{50}) according to Cheng and Prusoff [23], with a K_d value for NMS of 0.202 nM [18].

RESULTS

Effects of Propofol and Thiopentone on [^{3}H]NA Release

[^{3}H]Noradrenaline taken up into SH-SY5Y cells appears in a releasable pool with >95% as noradrenaline (measured by HPLC, [16]). Release can be evoked by both extracellular Ca^{2+}-dependent K^+ depolarization and extracellular Ca^{2+}-independent muscarinic M_3 receptor occupation (10, 14, 15). Basal, K^+-(100 mM), and carbachol (1 mM)-evoked release amounted to 3.5 ± 0.3% (n = 30), 13.3 ± 1.2% (n = 21), and 7.3 ± 0.8% (n = 24), respectively, K^+-evoked release was around 2-fold greater in magnitude than carbachol. Acute addition (3 min) of propofol or thiopentone

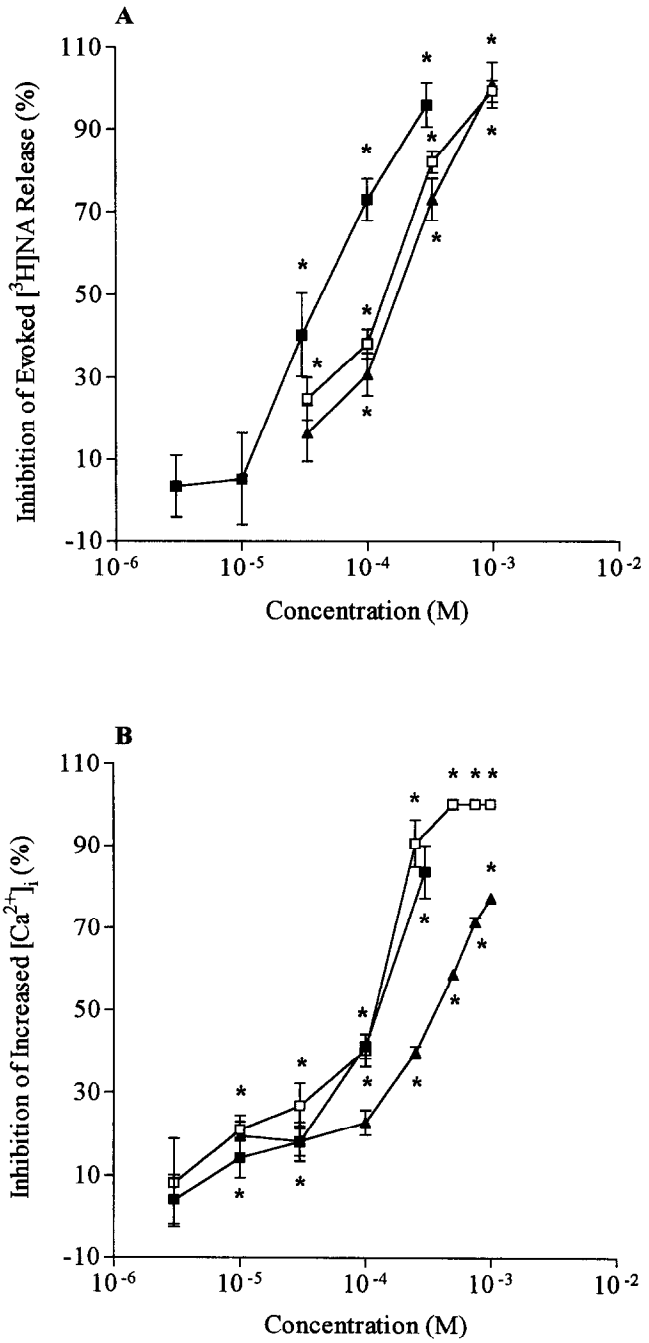

FIG. 1. Effects of propofol and thiopentone on: A. K^+-(100 mM) and carbachol-(1 mM) evoked [^{3}H]noradrenaline release, and B. K^+-(80 mM) and carbachol-(1 mM) stimulated increased intracellular Ca^{2+} concentrations from SH-SY5Y human neuroblastoma cells. ■ Represents K^+ plus propofol, □ represents K^+ plus thiopentone, and ▲ represents carbachol plus thiopentone. Data are mean ± SEM (n = 5–6). *P < 0.05 indicates significant inhibition compared with control. IC_{50} values for the inhibition of K^+-stimulated [^{3}H]NA release and increased $[Ca^{2+}]_i$ were 116 ± 15 and 121 ± 15 μM for thiopentone, and 42 ± 11 and 127 ± 7 μM for propofol, respectively. Thiopentone inhibited carbachol-stimulated stimulated [^{3}H]NA release and increased $[Ca^{2+}]_i$ with IC_{50} values of 169 ± 39 and 334 ± 30 μM, respectively.

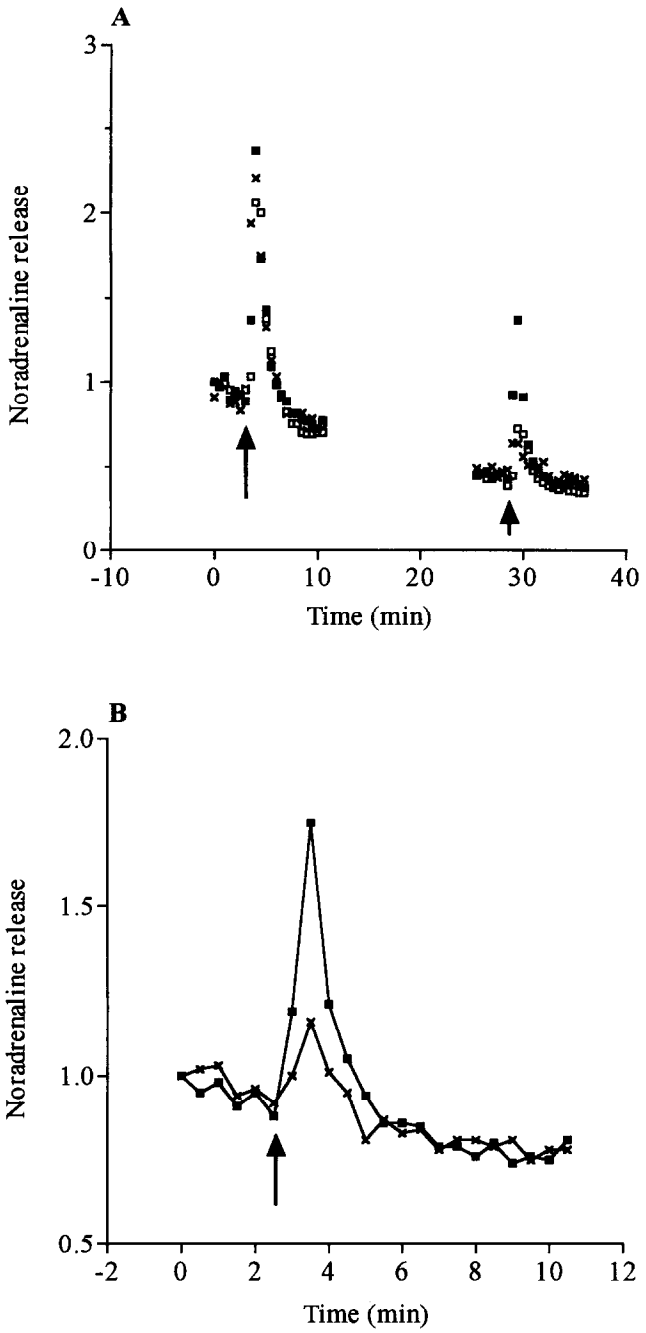

FIG. 2. Effects of propofol (□) and thiopentone (X) on (A) K^+-evoked and thiopentone on (B) carbachol-evoked [^{3}H]noradrenaline release. The control (i.e. no propofol or thiopentone) is represented by ■. In (A) propofol (100 μM) and thiopentone (333 μM) were added during the 15-min period between two 3-min pulses of K^+ (100 mM), S_1 and S_2, S_2/S_1 ratios were calculated. For carbachol-evoked release, thiopentone was added 15 min prior to S_1. Data are from a single experiment from n = 4–6 experiments, and are expressed relative to the first fraction collected (= 1.0). Stimulus addition is noted by the arrow.

did not affect basal release of [^{3}H]noradrenaline (data not shown). In addition, DMSO 0.003%–3% did not affect basal release (data not shown).

Propofol at 100 μM or 300 μM did not affect carbachol-evoked [^{3}H]noradrenaline release (data not shown), but this agent did cause a dose-dependent inhibition of K^+-evoked release, with significant inhibition occurring from 30 μM, and an IC_{50} of 42 ± 11 μM (Fig. 1A). In contrast to propofol, thiopentone produced a dose-dependent inhibition of both carbachol- and K^+-evoked [^{3}H]noradrenaline release (Fig. 1A), with significant inhibition occurring from 100 μM and 33 μM, respectively. The IC_{50} values for thiopentone inhibition of carbachol- and K^+-evoked release were not significantly different (Fig. 1A).

Further studies to determine if propofol and thiopentone altered the dynamics of [^{3}H]noradrenaline release were performed using a perfusion system. Both carbachol and K^+ stimulate a monophasic release of [^{3}H]noradrenaline with K^+ evoking a greater release measured by comparing areas under the curves, (Fig. 2). The effects of propofol (100 μM, > IC_{50}) and thiopentone (333 μM, > IC_{50}) on K^+-evoked release were assessed by calculating the S_2/S_1 (from area under the curve) ratio for a pair of pulses of 100 mM K^+. In view of the potential for receptor-mediated desensitization with carbachol, a single challenge was used. As can be clearly seen in (Fig. 2 and Table 1), both thiopentone and propofol reduced the S_2/S_1 ratio without affecting the dynamics of the S_2 release profile. In addition, thiopentone significantly reduced carbachol-evoked release without altering the release dynamics (Fig. 2 and Table 1).

Effects of Thiopentone on Muscarcinic Binding

It is also possible that thiopentone inhibition of carbachol-evoked [^{3}H]noradrenaline release could result from muscarinic-receptor blockade. Thiopentone caused a significant dose-dependent inhibition of [^{3}H]NMS binding to whole SH-SY5Y cells. Although 100% inhibition was not achieved (57% at 1 mM), based on a 100% inhibition, a K_i of 277 ± 7 μM (n = 4) was estimated. Using the Cheng-Prusoff equation [23], it can be calculated that 7.6 mM thiopentone would be required to inhibit a 1-mM carbachol response by 50%.

Effects of Propofol and Thiopentone on $[Ca^{2+}]_i$

To examine the mechanism of propofol and thiopentone inhibition of [^{3}H]noradrenaline release, intracellular Ca^{2+}

TABLE 1. Effects of propofol and thiopentone on [^{3}H]noradrenaline release from perfused cells

	A. Potassium, 100 mM		B. Carbachol, 1 mM	
	S_2/S_1 Ratio	n	Normalised AUC (arbitrary units)	n
Control	0.67 ± 0.07	5	1.72 ± 0.21	6
+100 μM Propofol	0.34 ± 0.03*	4		
+333 μM Thiopentone	0.19 ± 0.03*†	4	0.97 ± 0.10*	5

All data are mean ± SEM. AUC, area under the curve. * $P < 0.05$ reduced compared with control. † $P < 0.05$ lower than propofol.

concentrations were measured using the Ca^{2+}-sensitive dye Fura2, following K^+ or carbachol challenge. K^+ produced a time- and dose-dependent increase in $[Ca^{2+}]_i$ peaking at 9 sec, with an EC_{50} of 33 ± 1 mM (Fig. 3). Both thiopentone and propofol dose-dependently inhibited peak phase K^+-stimulated Ca^{2+} influx (Fig. 1B), with significant inhibition from 10 μM. The IC_{50} values for inhibition of peak phase K^+-stimulated Ca^{2+} influx were 121 ± 10 μM and 127 ± 7 μM for thiopentone and propofol, respectively (Fig. 1B). Thiopentone, but not propofol, inhibited the plateau phase of K^+-stimulated Ca^{2+} influx, completely abolishing the peak phase at concentrations above 250 μM (data not shown). Neither thiopentone nor propofol inhibition of peak phase K^+-stimulated Ca^{2+} influx was reversed by increasing concentrations of K^+, suggesting noncompetitive inhibition (Fig. 4A and B). There was no significant inhibition in Ca^{2+} influx in the presence or absence of GABA (data not shown), indicating that SH-SY5Y cells are unlikely to express $GABA_A$ receptors.

Propofol (100 μM) had no effect on carbachol-stimulated increased intracellular Ca^{2+} concentrations (data not shown). In contrast, thiopentone produced a dose-dependent inhibition of carbachol-stimulated intracellular Ca^{2+} concentrations, with significant inhibition at 250 μM and IC_{50} of 334 ± 30 μM (Fig. 1B). In the absence of Ca^{2+}, the basal and carbachol peak phase $[Ca^{2+}]_i$ were reduced and the carbachol plateau phase was essentially abolished (Fig. 5B). Thiopentone (250 and 500 μM) also inhibited increased intracellular Ca^{2+} concentrations stimulated by carbachol (100 μM) in Ca^{2+}-free buffer (Fig. 5B). Inhibition of carbachol-stimulated increased Ca^{2+} concentrations by thiopentone (300 μM) were not reversed by increasing doses of carbachol (1 μM to 1 mM, Fig. 6); again, suggesting noncompetitive inhibition. Thiopentone also produced noncompetitive inhibition of carbachol-stimulated plateau phase of Ca^{2+} entry (Fig. 6). Surprisingly, thiopentone had no effect on basal or carbachol-stimulated peak $Ins(1,4,5)P_3$ formation (Fig. 7).

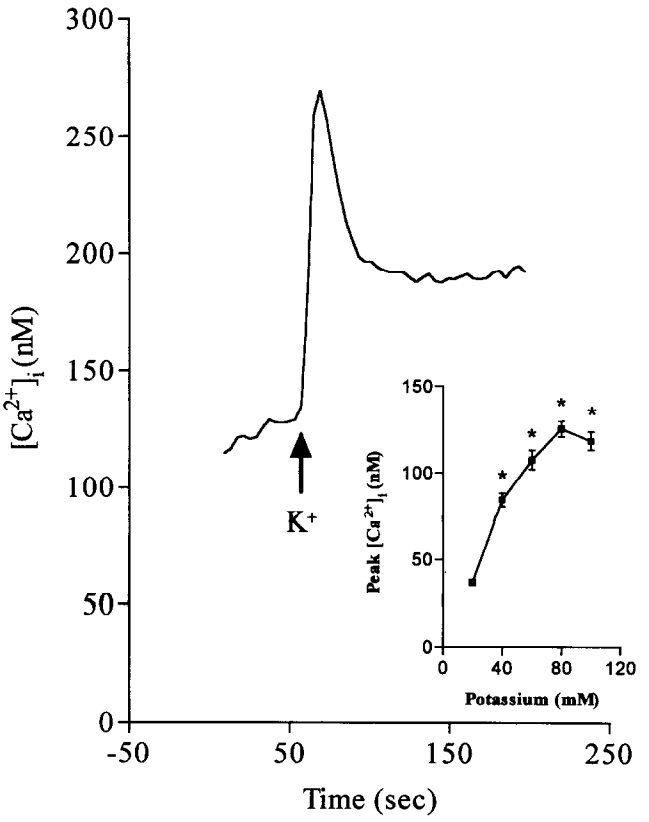

FIG. 3. The trace shows a typical biphasic response to K^+ (100 mM) with transient peak and sustained plateau. K^+ was added at 60 sec, as indicated by the arrow. The inset figure shows the change in $[Ca^{2+}]_i$ measured at the peak, after the addition of increasing concentrations of K^+. The data are expressed as the mean ± SEM, for n = 6 experiments. *Indicates $P < 0.05$ when compared to basal $[Ca^{2+}]_i$ in the absence of added K^+.

DISCUSSION

In this study, we demonstrate a differential effect of two commonly used intravenous anaesthetic agents on [^{3}H]noradrenaline release in an isolated human neuronal cell line of noradrenergic origin [9–11, 13].

We have previously shown that the release process from SH-SY5Y cells is stimulus-dependent. K^+-evoked [^{3}H]noradrenaline release is dependent on Ca^{2+} influx through voltage-sensitive Ca^{2+} channels that can be inhibited by the addition of Ni^{2+}, and carbachol-evoked release is driven through the release of Ca^{2+} from intracellular stores [16]. Further evidence from Murphy *et al.* [11] showed that carbachol-evoked release of [^{3}H]noradrenaline is inhibited by EGTA pretreatment in a time-dependent manner (i.e. possibly by causing a time-dependent depletion of the intracellular $Ins(1,4,5)P_3$-sensitive Ca^{2+} pools). In addition, Purkiss *et al.* [17] have demonstrated that the combination of low extracellular Ca^{2+} and depletion of $Ins(1,4,5)P_3$-sensitive stores with thapsigargin abolished both bradyinin- and methacholine-evoked [^{3}H]noradrenaline release. These data confirm the importance of $Ins(1,4,5)P_3$-evoked Ca^{2+} release from intracellular stores during muscarinic receptor-mediated [^{3}H]noradrenaline release. Therefore, although the inhibition of noradrenaline release may not be the determining factor involved in the process of anaesthesia, this well-characterized model allows detailed examination of the interaction of anaesthetic agents with stimulus secretion coupling.

Propofol inhibited depolarization (K^+)-evoked [^{3}H]noradrenaline release without affecting agonist (carbachol)-driven release. Furthermore, propofol inhibited K^+-stimulated peak phase Ca^{2+} influx after 15-min preincubation, but did not affect the sustained phase when added acutely after K^+ challenge, or carbachol-stimulated increased $[Ca^{2+}]_i$. In addition, we have previously shown that propofol had no effect on carbachol-stimulated $Ins(1,4,5)P_3$ formation [24]. These data suggest that propofol inhibits the functioning of voltage-sensitive Ca^{2+} channels. More-

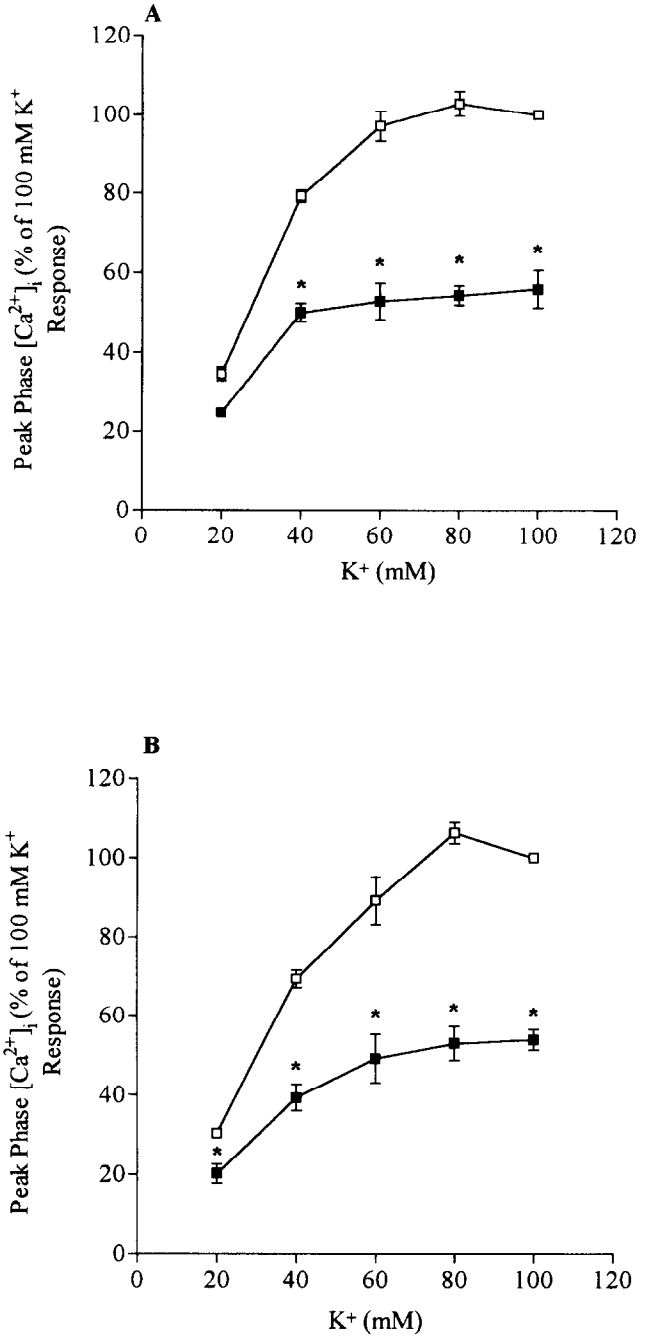

FIG. 4. K^+ (20 to 100 mM added) dose-response curves in the presence (■) or absence (□) of (A) propofol (100 μM) or (B) thiopentone (100 μM). Dose-response curves run in the absence of propofol or thiopentone contained an equivalent concentration of the appropriate control (i.e. DMSO or NaOH, respectively). Data are expressed as a percentage of the maximum response, and are mean ± SEM, for n = 5 to 6 experiments. *Indicates $P < 0.05$ when compared to the control. The EC_{50} values for K^+ stimulation of $[Ca^{2+}]_i$ increase was 31 ± 0.7 and 28 ± 0.8 mM for control and thiopentone, and 34 ± 0.9 and 34 ± 3.0 mM for control and propofol, respectively.

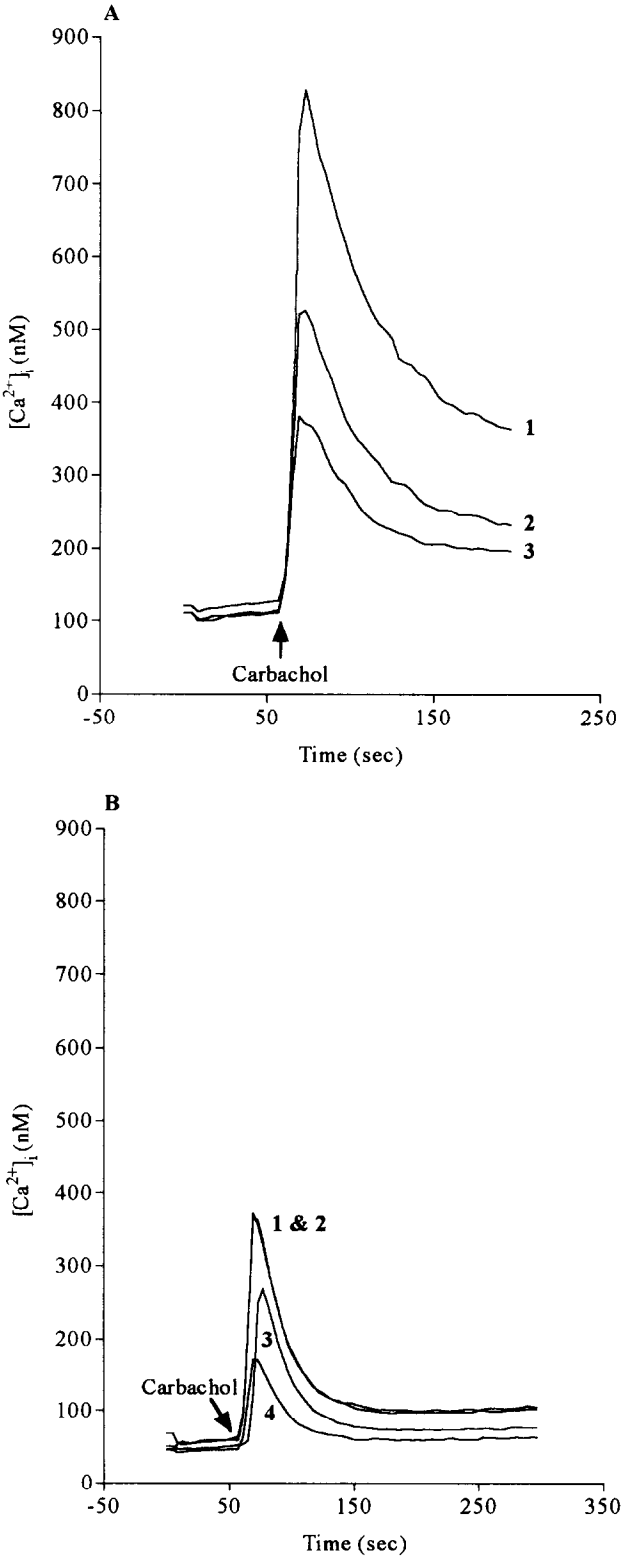

FIG. 5. A. Effects of thiopentone on carbachol-stimulated (100 μM) increased intracellular Ca^{2+} concentrations after 15-min preincubation with either NaOH (trace 1), or thiopentone (250 μM, trace 2; 500 μM, trace 3). All 3 traces are typical of 5 others. B. Inhibition of carbachol-stimulated increased intracellular Ca^{2+} concentrations by thiopentone (250 μM, trace 3; or 500 μM trace 4) after 15-min preincubation in the absence of extracellular Ca^{2+}. Traces 1 and 2 show typical carbachol responses after 15-min preincubation with an equivalent concentration of the vehicle, NaOH, used when either 250 or 500 μM thiopentone was added. The traces shown are representative of 4 others for each concentrations examined.

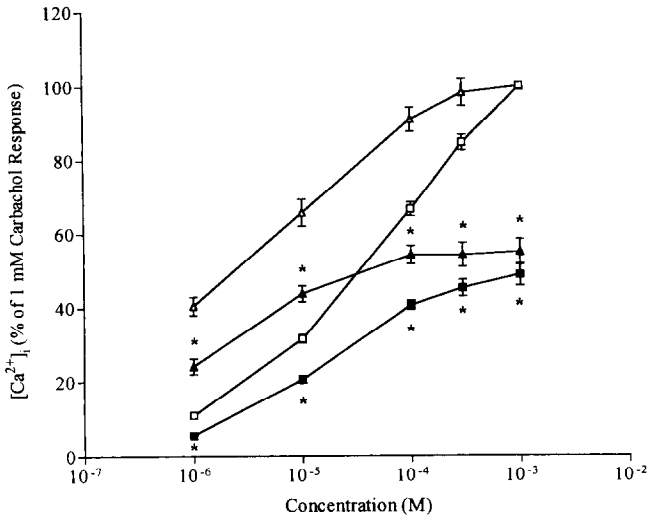

FIG. 6. Carbachol (1 μM to 1 mM) dose-response curve after 15-min preincubation in the presence or absence of thiopentone (300 μM). In the absence of thiopentone, an equivalent concentration of the vehicle, NaOH, was added 15 min before carbachol challenge. Carbachol peak phase in the absence and presence of thiopentone is □ and ■, respectively. Carbachol plateau phase in the absence and presence of thiopentone is denoted by △ and ▲, respectively. The EC_{50} values for the peak phase were 50 ± 4.7 and 23 ± 2.5 μM for control and thiopentone. For the plateau phase, the EC_{50} values were 19 ± 3.8 μM for control and 9 ± 0.9 μM for thiopentone. Data are expressed as the percentage of the maximum carbachol response in the absence of thiopentone, and are mean ± SEM, for $n = 6$ experiments. *Indicates $P < 0.05$ when compared to the absence of thiopentone (by Student's paired t-test).

over, the inhibition of Ca^{2+} entry was not reversed by increasing the dose of K^+, suggesting that propofol inhibition of Ca^{2+} influx is noncompetitive. We have made similar preliminary observations with the noncompetitive NMDA antagonist ketamine [25].

There have been very few direst electrophysiological studies of the effects of propofol on Ca^{2+} ion channels. Propofol at 50 μM had no effect on the function of isolated guinea-pig papillary muscle sarcoplasmic reticulum [26]. However, at 100 μM, this agent did alter the kinetics of guinea-pig ventricular myocyte L-type voltage-sensitive Ca^{2+} channels, to favour the closed state without affecting conductance [27]. In addition, in chick-embryo dorsal root ganglion neurones, propofol (300 μM) reduced the T-type voltage-sensitive Ca^{2+} channel by around 80% and the L-type voltage-sensitive Ca^{2+} channel by around 75%. N-type channels appeared relatively unaffected [28]. Unfortunately, very high concentrations of omega-conotoxin (5 μM) and nifedipine (10 μM) were used to attempt to discriminate between N and L-type currents. Peak serum propofol concentrations seen during the induction of anaesthesia are around 35 μM [29], but the free drug concentration would be lower. The IC_{50} for inhibition of K^+-evoked [^{3}H]noradrenaline release in this study (42 μM) compares favourably with total serum propofol concentrations. In addition, significant inhibition of K^+-stimulated Ca^{2+} influx was achieved with concentrations of propofol as low as 10 μM. However, the contribution of propofol binding to SH-SY5Y cells may significantly lower the free concentration.

Peak serum concentrations of thiopentone on induction of anaesthesia are around 380 μM [30] (but will be reduced by protein binding); again, the contribution of SH-SY5Y cell protein to the free drug concentration has not been determined. However, this value (380 μM) compares favourably with the IC_{50} values for inhibition of K^+ and carbachol-evoked [^{3}H]noradrenaline release and increased intracellular Ca^{2+}. Haycock *et al.* [31] demonstrated that pentobarbitone inhibited K^+- and veratridine-evoked [^{3}H]noradrenaline release from synaptosomes, but had no effect on A23187-facilitated release. These data provided preliminary evidence that barbiturates blocked voltage-sensitive Ca^{2+} channels. Moreover, Pocock and Richards [32] showed that pentobarbitone inhibited K^+-evoked catecholamine release from bovine adrenal chromaffin cells, and blocked $^{45}Ca^{2+}$ entry; because catecholamine secretion was proportional to the rise in intracellular $^{45}Ca^{2+}$, they concluded that pentobarbitone blocked voltage-sensitive Ca^{2+} channels. There are few direct electrophysiological studies in the literature to support this supposition [33, 34] but Park and Lynch [35] recently demonstrated in guinea-pig ventricular myocytes that thiopentone inhibited voltage-sensitive Ca^{2+} channels. Further evidence that barbiturates may modulate the release of intracellular Ca^{2+} was provided by Deshmukh *et al.* [36], who demonstrated that

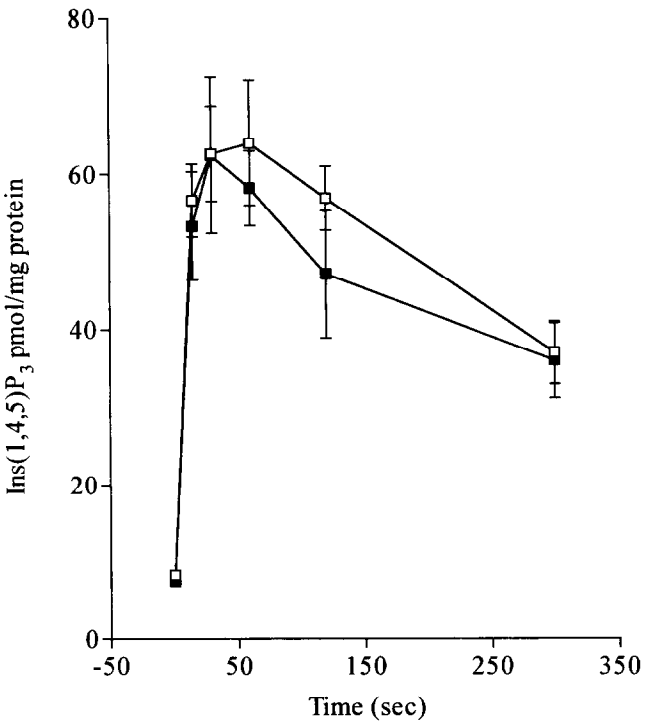

FIG. 7. Effects of thiopentone (100 μM, ■) or the vehicle, NaOH (□) on Ins(1,4,5)P_3 formation after carbachol (100 μM) challenge. Data are expressed as mean ± SEM, for $n = 5$ experiments.

these agents inhibited the receptor-mediated rise in intracellular Ca^{2+} in platelets that originated from both entry and store release.

Thiopentone inhibits both K^+- and carbachol-evoked [^{3}H]noradrenaline release equally, suggesting that thiopentone interferes with both Ca^{2+} influx and release from intracellular stores (see above and [16]). Indeed, thiopentone inhibits both K^+- and carbachol-stimulated increases in intracellular Ca^{2+} concentrations. Inhibition of K^+-stimulated Ca^{2+} influx infers that thiopentone inhibits voltage-sensitive Ca^{2+} channels. It is unclear, in this cell line, which voltage-sensitive channels are affected by thiopentone or propofol; however, because both peak and plateau phases of Ca^{2+} influx are reduced, both N- and L-type calcium channels may be inhibited [37–39]. Further investigation to discriminate between the contribution of voltage-sensitive Ca^{2+} channel types is required.

We have demonstrated, using SH-SY5Y cells, that carbachol-stimulated increases in intracellular Ca^{2+} concentrations are *via* agonist-induced Ins(1,4,5)P_3 formation, and subsequent release of Ca^{2+} from Ins(1,4,5)P_3-sensitive intracellular stores [40]. This transient rise in intracellular Ca^{2+} concentrations is followed by a sustained increase *via* Ca^{2+} entry [40]. In this cell line, carbachol-stimulated Ca^{2+} entry is not attenuated by the addition of dihydropyridines or ω-conotoxin, suggesting that the sustained phase of Ca^{2+} entry is not mediated through voltage-sensitive Ca^{2+} channels [19], but *via* a receptor-operated calcium channel [40]. The reported difference in EC_{50} for peak and plateau phases of carbachol-stimulated increased $[Ca^{2+}]_i$, with the plateau phase being more potent, is confirmed in this study.

Previous investigations have shown that pentobarbitone inhibited carbachol- and substance P-stimulated [^{3}H]total inositol phosphate formation [41] and a range of other barbiturates inhibit angiotensin II-stimulated [^{3}H]total inositol phosphate formation [42]. These agents also inhibit the biosynthetic pathway for phosphoinositides by interacting with phophatylinositol-4-phosphate and phosphatidylinositol kinases [43, 44]. Because carbachol-evoked [^{3}H]noradrenaline release is dependent on release of Ca^{2+} from Ins(1,4,5)P_3-sensitive stores [16], thiopentone may inhibit Ins(1,4,5)P_3 formation. Surprisingly, these data show that thiopentone did not effect carbachol-stimulated Ins(1,4,5)P_3 formation. It is possible that thiopentone could interact at a site downstream of Ins(1,4,5)P_3 formation. This may be at the site of Ca^{2+} release from intracellular stores, because thiopentone inhibited carbachol-stimulated rises in intracellular Ca^{2+} in the presence or absence of extracellular Ca^{2+}.

As the inhibition of K^+- and carbachol-stimulated [^{3}H]NA release by thiopentone occurs with similar IC_{50}, values it could be argued that thiopentone is interacting with the release machinery. However, as there is a similar dose-related inhibition in K^+-evoked Ca^{2+} influx and carbachol-evoked release of intracellular stores (that control the release process [16, 17]) this is unlikely.

A potential explanation for thiopentone or propofol inhibition of K^+-stimulated Ca^{2+} influx may be due to the well-characterised [4] interaction with $GABA_A$ receptors to produce hyperpolarization. The failure of GABA (100 μM) to inhibit K^+-stimulated Ca^{2+} influx despite 15-min preincubation suggests that, if GABA receptors exist in SH-SY5Y cells they are not functionally coupled, and that the observed inhibition is not due to $GABA_A$ receptor interaction.

An alternative explanation for the inhibitory effect of thiopentone on carbachol-evoked release may result from an interaction with protein kinase C, which as been shown to enhance [^{3}H]noradrenaline release in SH-SY5Y cells [12]. In a recent study, Slater *et al.* [45] reported that the volatile anaesthetic agents halothane and enflurane, as well as ethanol, directly inhibit protein kinase C. Whether or not barbiturates have similar actions remains to be determined.

In summary, we report a differential inhibition of K^+- and carbachol-evoked [^{3}H]noradrenaline release by propofol and thiopentone. The "selective" inhibition of K^+-evoked release and K^+-stimulated Ca^{2+} influx by propofol suggests that this agent may act as a Ca^{2+} channel blocker. Thiopentone may not only act as a voltage-sensitive Ca^{2+} channel blocker, but inhibits agonist-driven increases in intracellular Ca^{2+} to prevent carbachol-evoked [^{3}H]noradrenaline release. This inhibition is not due to reduced Ins(1,4,5)P_3 formation, and suggest that thiopentone interferes with the release of Ca^{2+} from intracellular stores.

R. Atcheson was a British Journal of Anaesthesia Research Fellow. We thank Rhone-Poulenc Rorer, U.K. for providing thiopentone and Zeneca Pharmaceuticals, U.K. for providing pure propofol.

References

1. Kress HG, Effects of general anaesthetics on 2nd messenger systems. *E.J. Anaesthesiology* **12:** 83–99, 1995.
2. Pocock G and Richards CD, Excitatory and inhibitory synaptic mechanisms in anaesthesia. *Brit J Anaes* **71:** 134–147, 1993.
3. Halsey MJ, (1991) How do intravenous anaesthetics work? In: *Bailliere's Clinical Anesthesiology* (Eds. Dundee, J. W. and Sear, J. W.), pp Vol 5(2) Intravenous Anaesthesia-What is New Vol 5(2).
4. Franks NP, Lieb WR, Molecular and cellular mechanisms of general anaesthesia. *Nature* **367:** 607–614, 1994.
5. Waller MB and Richter JA, Effects of pentobarbital and Ca^{2+} on the resting and K^+-stimulated release of several endogenous neurotransmitters from rat midbrain slices. *Biochem Pharmacol* **29:** 2189–2198, 1980.
6. Lecharny J-B, Salord F, Henzel D, Desmonts J-M and Mantz J, Effects of thiopental, halothane and isofluorane on the calcium-dependent and -independent release of GABA from striatal synpatosomes in the rat. *Brain Res* **670:** 308–312, 1995.
7. Griffiths R and Norman RI, Effects of anaesthetics on uptake, synthesis and release of transmitters. *Brit J Anaes* **71:** 96–107, 1993.
8. Leidenheimer NJ, Activation of calcium-phospholipid-dependent protein kinase enhances benzodiazepine and barbiturate potentiation of $GABA_A$ receptors. *J Neurochem* **61:** 1479–1491, 1993.

9. Ross RA, Spengler BA and Biedler JL, Coordinate morphological and biochemical interconversion of human neuroblastoma cells. *J Natl Cancer Inst* **71:** 741–749, 1983.
10. Ross RA and Biedler JL, Presence and regulation of tyrosinase activity in human neuroblastoma cell variants in vitro. *Cancer Res* **45:** 1628–1632, 1985.
11. Murphy NP, Ball SG and Vaughan PFT, Potassium- and carbachol-evoked release of [^{3}H]noradrenaline from human neuroblastoma cells, SH-SY5Y. *J Neurochem* **56:** 1811–1815, 1991.
12. Murphy NP, McCormack JG, Ball SG and Vaughan PFT, The effect of protein kinase C activation on muscarinic-M_3- and K^+-evoked release of [^{3}H]noradrenaline and increases in intracellular Ca^{2+} in human neuroblastoma SH-SY5Y cells. *Biochem J* **282:** 645–650, 1992.
13. Scott IG, Akerman KEO, Heikkila JE, Kaila K and Andersson LC, Development of a neural phenotype in differentiating ganglion cell-derived human neuroblastoma cells. *J Cell Physiol* **128:** 285–292, 1986.
14. Atcheson R, Rowbotham DJ and Lambert DG, Fentanyl inhibits the uptake of [^{3}H]noradrenaline in cultured neuronal cells. *Brit J Anaes* **71:** 540–543, 1993.
15. Atcheson R, Rowbotham DJ and Lambert DG, Fentanyl inhibits the release of [^{3}H]noradrenaline from SH-SY5Y human neuroblastoma cells. *Brit J Anaes* **72:** 98–103, 1994.
16. Atcheson R, Lambert DG, Hirst RA and Rowbotham DJ, Studies on the mechanism of [^{3}H]-noradrenaline release from SH-SY5Y cells: the role of Ca^{2+} and cyclic AMP. *Brit J Pharmacol* **111:** 787–792, 1994.
17. Purkiss JR, Nahorski SR and Willars GB, Mobilization of inositol 1,4,5-triphosphate-sensitive Ca^{2+} stores supports bradykinin- and muscarinic-evoked release of [^{3}H]noradrenaline from SH-SY5Y cells. *J Neurochem* **64:** 1175–1182, 1995.
18. Lambert DG, Ghataorre AS and Nahorski SR, Muscarinic receptor binding characteristics of a human neuroblastoma SK-N-SH and its clones SH-SY5Y and SH-EP1. *Eur J Pharmacol* **165:** 71–77, 1989.
19. Lambert DG, Whitham EM, Baird JG and Nahorski SR, Different mechanisms of Ca^{2+} entry induced by depolarization and muscarinic receptor stimulation in SH-SY5Y human neuroblastoma cells. *Mol Brain Res* **8:** 263–266, 1990.
20. Grynkiewicz G, Poenic M, Tsien RY, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* **260:** 3440–3449, 1985.
21. Challis RAJ, Batty IH and Nahorski SR, Mass measurement of inositol (1,4,5) triphosphate in rat cerebral cortex slices using a radioreceptor assay: effects of neurotransmitters and depolarization. *Biochem Biophys Res Commun* **157:** 684–691, 1988.
22. Smart D, Smith G and Lambert DG, μ-Opioids activate phospholipase C in SH-SY5Y cells via calcium-channel opening. *Biochem J* **305:** 577–582, 1995.
23. Cheng YC and Pursoff WH, Relationship between the inhibitor constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22:** 3099–3108, 1973.
24. Cembala TM, Frost CL, Atcheson R, Hirst RA, Smart D and Lambert DG, The effects of propofol on cAMP and Ins(1,4,5)P_3 formation and [^{3}H]noradrenaline uptake and release from SH-SY5Y cells. *Biochem Soc Trans* (Abstr) **22:** 290S, 1994.
25. Sikand KS, Smith G and Lambert DG, Ketamine inhibits K^+ evoked [^{3}H]noradrenaline release from SH-SY5Y cells by reducing calcium influx. *Biochem Soc Trans* **23:** 417S, 1995.
26. Puttick RM and Terrar DA, Differential effects of propofol and enflurane on contractions dependent on calcium derived from the sarcoplasmic reticulum of guinea pig isolated papillary muscles. *Anaes Analg* **77:** 55–60, 1993.
27. Takahashi H, Puttick RM and Terrar DA, The effects of propofol and enflurane on single calcium channel currents of guinea-pig isolated ventricular myocytes. *Brit J Pharmacol* **111:** 1147–1153, 1994.
28. Olcese R, Usai C, Maestrone E and Nobile M, The general anaesthetic propofol inhibits transmembrane calcium current in chick sensory neurones. *Anaes Analg* **78:** 955–960, 1994.
29. Shafer A, Doze VA, Shafer SL and White PF, Pharmacokinetics and pharmacodynamics of propofol infusions during general anaesthesia. *Anesthesiology* **69:** 348–356, 1988.
30. Frenkel C, Duch DS and Urban BW, Effects of I.V. anaesthetics on human brain sodium channels. *Brit J Anaes* **71:** 15–24, 1993.
31. Haycock JW, Levy WB and Cotman CW, Pentobarbital depression of stimulus-secretion coupling in brain; selective inhibition of depolarization-induced calcium-dependent release. *Biochem Pharmacol* **26:** 159–161, 1977.
32. Pocock G and Richards CD, The action of pentobarbitone on stimulus-secretion coupling in adrenal chromaffin cells. *Brit J Pharmacol* **90:** 71–80, 1987.
33. Charlesworth P, Pocock G and Richards CD, Calcium-channel currents in bovine adrenal chromaffin cells and their modulation by anaesthetic agents. *J Physiol* **481:** 543–553, 1994.
34. Pancrazio JJ, Park WK and Lynch C, Inhalational anaesthetic actions on voltage-gated ion currents of bovine adrenal chromaffin cells. *Mol Pharmacol* **43:** 783–794, 1993.
35. Park WK and Lynch C, Propofol and thiopental depression of myocardial contractility; a comparative study of mechanical and electrophysiologic effects in isolated guinea pig ventricular muscle. *Anaes Analg* **74:** 395–405.
36. Deshmukh DS, Kuizon S and Brockerhoff H, Barbiturates inhibit intracellular Ca^{2+} rise induced by thrombin in rat platelets. *Cell Calcium* **12:** 645–654, 1991.
37. Tosseli M, Massetto S, Rossi P and Taglietti V, Characterisation of a voltage-dependent calcium current in the human neuroblastoma cell line SH-SY5Y during differentiation. *Eur J Neurosci* **3:** 514–522, 1991.
38. Morton AJ, Hammond C, Mason W and Henderson G, Characterisation of the L- and N-type calcium channels in differentiated SH-SY5Y neuroblastoma cells: calcium imaging and single channel recording. *Mol Brain Res* **13:** 53–61, 1992.
39. McDonald RL, Vaughan PFT and Peers C, Muscarinic (M_1) receptor-mediated inhibition of K^+ release from human neuroblastoma (SH-SY5Y) cells via inhibition of L- and N-type Ca^{2+} channels. *Brit J Pharmacol* **113:** 621–627, 1994.
40. Lambert DG and Nahorski SR, Muscarinic-receptor-mediated changes in intracellular Ca^{2+} and insoitol 1,4,5-triphosphate mass in a human neuroblastoma cell line, SH-SY5Y. *Biochem J* **265:** 555–562, 1990.
41. Hassessian H, Prat A and Couture R, Anaesthetic doses of pentobarbital antagonize phosphatidylinositol hydroysis by substance P or carbachol in the spinal cord and cerebral cortex of the rat. *Eur J Pharmacol* **227:** 103–107, 1992.
42. Robinson-White AJ, Muldoon SM and Robinson FC, Inhibition of inositol phosphate hydrolysis in endothelial cells by pentobarbital. *Eur J Pharmacol* **172:** 291–303, 1989.
43. Deshmukh KS, Kuizon S and Brockerhoff H, Effect of phenobarbital on metabolism of polyphosphoinositides of rat brain synaptosomes. *Life Sci* **41:** 2121–2126, 1987.
44. Deshmukh DS, Kuizon S, Chauhan VPS and Brockerhoff H, Effect of barbiturates on polyphosphoinositide biosynthesis and protein kinase C activity. *Neuropharmacology* **28:** 1317–1323, 1989.
45. Slater SJ, Cox KJA, Lombardi JV, Ho C, Kelly MB, Rubin E and Stubbs CD, Inhibition of protein kinase C by alcohols and anaesthetics. *Nature* **64:** 82–84, 1993.