Clin. exp. Immunol. (1982) **47**, 457–466.

Differential effects of intravenous anaesthetic agents on cell-mediated immunity in the Rhesus monkey

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(Accepted for publication 7 August 1981)

SUMMARY

Considerable evidence has accumulated to implicate general anaesthetic agents as a cause of post-surgical immune depression. In the present study we evaluated the immunosuppressive effects of three *in vivo* administered anaesthetic agents on cellular immune function in sub-human primates which did not undergo surgery. Normal rhesus monkeys received a minimal anaesthetic dose of ketamine HCl, meperidine HCl, or sodium pentobarbital. Peripheral blood mononuclear cells were assayed for mitogen-induced lymphocyte proliferative responses and cell-mediated cytotoxicity (CC), including antibody-dependent CC, spontaneous CC and alloimmune CC. *In vivo* administration of the three agents caused significant reduction in lymphocyte functional capabilities. Within 30 min after administration of ketamine HCl or sodium pentobarbital, cytotoxic effector function was significantly depressed, with variable recovery occurring at 48 hr; cytolytic effector function was not impaired after meperidine HCl or in untreated controls. Ketamine HCl selectively suppressed effector function; mitogen-induced lymphocyte proliferative responses were not suppressed. Monkeys given meperidine HCl showed stable effector function and depressed lymphocyte proliferative function. Effects from sodium pentobarbital were non-selective, with reduced cytotoxic and proliferative lymphocyte functions. In summary, this study shows that intravenous anaesthetic agents are immunosuppressive in primates and exhibit disparate effects on afferent and efferent expressions of cellular immunity.

INTRODUCTION

Considerable evidence indicates that adverse alteration in host immunocompetence follows surgical procedures performed under general anaesthesia. Graham (1911) first described inhibition of the phagocytic activity of human leucocytes by ether. Subsequently, suppression by various anaesthetic agents of macrophage and lymphocyte functions has been described, leading to speculation that post-surgical complications such as infection and tumour metastasis may be in part related to suppressive effects of anaesthetic agents on relevant host immune defence systems (Bruce *et al.*,

Abbreviations: LMC = antigen-induced lymphocyte-mediated cytotoxicity; ADCC = antibody-dependent cell-mediated cytotoxicity; SCC = spontaneous or natural cell-mediated cytotoxicity; PBMC = peripheral blood mononuclear cells; LU = lytic units; Con A = concanavalin A; cAMP = cyclic adenosine monophosphate; cGMP = cyclic guanosine monophosphate.

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0009-9104/82/0200-0457$02.00

1968; Bruce & Wingard, 1971; Duncan & Cullen, 1976; Moudgil & Wade, 1976; Whitwam & Norman, 1979).

Studies of surgical patients support this hypothesis, revealing depressions in B and T lymphocyte counts (Salo, 1978), impairment of lymphocyte blastogenesis (Berenbaum, Fluck & Hurst, 1973; Espanol, Todd & Soothill, 1974; Cooper, Irvine & Turnbull, 1974), and cytotoxic effector function (Kumar & Taylor, 1974; Vose & Moudgil, 1976; Micheels, Degiovanni & Castermans, 1978) after administration of volatile anaesthetic agents. But effects from such other factors as disease state, surgery, trauma, and medication have not been excluded. Moreover, conflicting data have challenged the concept that anaesthesia *per se* is immunosuppressive (Jubert *et al.*, 1973; Kanto, Vapaavuori & Viljanen, 1974).

In several experimental animal models with controlled variables, *in vivo* alterations of the immune response by anaesthesia have been described. For example, mice given halothane have higher death rates following viral (Moudgil, 1973) and bacterial infections (Bruce, 1967; Duncan, Cullen & Pearsall, 1976) than untreated controls. Rodent studies have shown modification of host response to neoplasia after anaesthesia, i.e. enhanced growth of spontaneous tumours (Peraino, Fry & Staffeldt, 1973; Van Den Brenk & Shapington, 1972) and chemically induced tumours (Peraino *et al.*, 1973). Studies in lower animals revealed depressed antibody formation (Humphrey, Wingard & Lang, 1969) and delayed allograft rejection (Eyal *et al.*, 1965) after anaesthesia. Most controlled studies have focused on a single *in vitro* or *in vivo* immune parameter, however, and mechanisms of impaired host immune resistance to infections and tumours following anaesthesia have not yet been clearly explained.

Here, we describe the *in vivo* immunosuppressive effects of three intravenous anaesthetic agents on several defined *in vitro* cellular immune parameters in normal rhesus monkeys. To eliminate any effects from surgery, the primates received anaesthesia only. The data show disparate immunosuppressive effects of these agents on afferent and efferent expressions of cell-mediated immunity.

MATERIALS AND METHODS

Animals. The animals were normal rhesus monkeys (Litton Bionetics, Yemassee, South Carolina) of both sexes weighing 3·5–8·0 kg. All were conditioned for 3 months and maintained according to USPHS regulations for care and handling of primates.

In vivo *administration of anaesthetic agents.* Four groups of animals were studied. Group 1 received an intermediate-acting barbiturate, sodium pentobarbital, 30 mg/kg (Diabutal, Diamonds Laboratories, Des Moines, Iowa). Group 2 received a narcotic, meperidine HCl, 10 mg/kg (Demerol, Wyeth Laboratories, Philadelphia, Pennsylvania). Group 3 received a dissociative anaesthetic, ketamine HCl, 10 mg/kg (Ketaject, Bristol Laboratories, Syracuse, New York). Each group was given a single parenteral dose, the minimum required for a surgical plane of anaesthesia. Group 4 were untreated controls.

In vitro *exposure to anaesthetic agents.* Blood samples containing preservative-free heparin, 10 units/ml (Chromalloy Pharmaceuticals, St Louis, Missouri) were incubated *in vitro* for 30 min at 37°C with either ketamine HCl or sodium pentobarbital. Ketamine HCl was added at serial 10-fold dilutions between 0·0013 and 1·3 mg/ml; sodium pentobarbital was added at 10-fold dilutions between 0·0039 and 3·9 mg/ml. The range of doses tested included the maximum possible *in vivo* concentration, assuming a mean rhesus blood volume of 77 ml/kg. After the 30-min incubation period, peripheral blood mononuclear cells were isolated and washed.

Peripheral blood mononuclear cell (PBMC) suspensions. Mononuclear cells were isolated on a Ficoll–Hypaque gradient (Pharmacia, Piscataway, New Jersey). PBMC at the interphase were collected and washed three times with culture medium, RPMI 1640 (GIBCO, Grand Island, New York), supplemented with 20 mM Hepes buffer (GIBCO), 2 mM L-glutamine (GIBCO), and 1% penicillin-streptomycin (GIBCO). Cell viabilities were assessed with trypan blue; only viable cells were counted to adjust the concentration of cell suspension. PBMC subpopulations of T lymphocytes, B lymphocytes and Fc-receptor-bearing lymphocytes were identified by their surface receptors for sheep RBC, complement, and IgG, respectively (Thomas *et al.*, 1979).

Cell-mediated cytotoxicity. Three types of cytotoxic activity were studied using the chromium-51-release technique (Thomas *et al.*, 1979). The assays were performed in microculture plates (Linbro, New Haven, Connecticut) in a final volume of 0·25 ml, in culture medium containing 20% fetal calf serum (GIBCO). After effectors and target cells had been mixed, the plates were centrifuged at 50 *g* for 5 min and incubated at 37°C for 4 hr in a humidified atmosphere of 95% air and 5% CO_2. Supernatants were collected by the Skatron Titertek system (Flow Labs, McLean, Virginia) and counted in a Beckman gamma counter. Spontaneous release of chromium-51 from target cells was assayed in the absence of effector cells and ranged between 8 and 20% of the maximum release obtained in the presence of a 4% Cetrimide solution (Sigma, St Louis, Missouri). The percentage of lysis was calculated by the formula: (experimental c.p.m. − spontaneous c.p.m./maximum c.p.m. − spontaneous c.p.m.) × 100. Within the range of effector/target ratios detailed below, the percentage of lysis varied linearly ($r > 0·9$) with the number of effector cells added. Data are expressed as percentage of lysis at the highest E/T ratio and as lytic units (LU) calculated as the number of effectors required to lyse 50% of the target cells.

Direct T-lymphocyte-mediated cytotoxicity (LMC). This was tested against specific allogenic donor lymphocytes to which the recipient had been immunized 2–3 weeks previously. Autologous targets served as specificity controls. Effector to target ratios were 75:1, 25:1 and 8:1 for each target.

Antibody-dependent cell mediated cytotoxicity (ADCC) and spontaneous or natural cell mediated cytotoxicity (SCC). These were assayed at the same time in normal nonimmunized animals. Target cells were normal human PBMC. For the ADCC assay, chromium-51 tagged targets were sensitized with the IgG fraction of rabbit antihuman lymphocyte globulin. Effector to target ratios were 25:1, 12:1 and 6:1.

Mitogen-induced lymphocyte proliferation. The proliferative responsiveness of rhesus PBMC taken before and serially after *in vivo* administration of anaesthetic agents was tested against a polyclonal T-cell mitogen, soluble concanavalin A (Con A) (Calbiochem, San Diego, California) and a B cell mitogen, soluble staphylococcal protein A (Pharmacia, Piscataway, New Jersey). The cells were cultured in triplicate in microculture trays (Linbro) at a concentration of $1·5 \times 10^5$ cells per well. Culture medium contained 20% pooled normal monkey serum to which mitogen in varying concentrations was added. Cultures were incubated for 72 hr, pulsed for 12 hr with 1 μCi ^{3}H-thymidine (New England Nuclear) per well, harvested with an automated cell harvester, and measured for radioactivity in a Beckman liquid scintillation counter. Results are presented as the mean peak c.p.m. obtained in the presence of mitogen minus the c.p.m. obtained in the media controls.

Cyclic nucleotide levels. Intracellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) were measured by radioimmunoassay according to the method of Steiner, Parker & Kipnis (1972). The cyclic nucleotides in trichloroacetic acid extracts of 1×10^6 rhesus lymphocytes were purified by Sephadex column chromatography, concentrated, and tested by assaying the displacement by unlabelled cyclic nucleotide of ^{125}I labelled tyrosine methyl ester-succinyl cyclic nucleotide from specific antibody.

Statistical analysis. The data were evaluated for statistical significance using a two-tailed Student's *t* test.

RESULTS

Changes in cytolytic effector cell activity

Within 30 min after administration of sodium pentobarbital or ketamine HCl, a 60% decline ($P < 0·025$) occurred in ADCC effector activity (Fig. 1). Reduction in SCC activity was significant within 30 min in animals given ketamine HCl ($P < 0·001$) and at 2 hr in those given sodium pentobarbital ($P < 0·05$). By 48 hr SCC activity had fully recovered, but ADCC effector activity was only partially recovered (to a mean 70% of the pretreatment level) in both groups. In contrast to these two groups, neither untreated controls nor monkeys given meperidine HCl showed statistically significant changes in cytolytic effector activity.

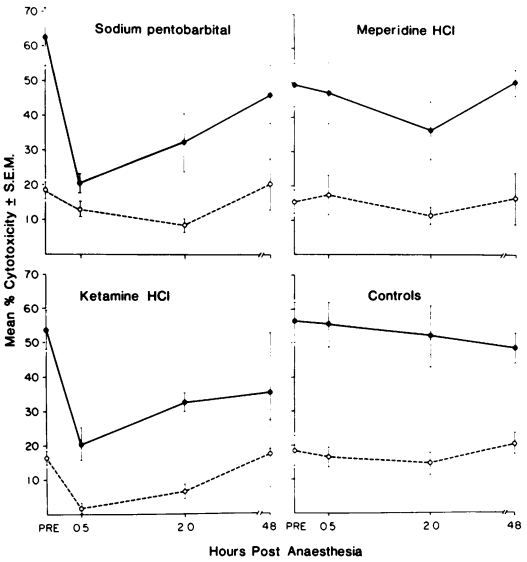

Fig. 1. Changes in cell-mediated cytotoxicity after *in vivo* administration of intravenous anaesthetic agents. ADCC and SCC activity of rhesus lymphocytes were tested against antibody coated and uncoated human lymphocyte target cells using a 4 hr chromium release assay. The data are expressed as the mean percent lysis ± s.e.m. obtained at an effector to target cell ratio of 25:1. The depression in ADCC or K cell (●——●) cytotoxicity is statistically significant at 30 min ($P < 0{\cdot}025$) and also at 2 hr ($P < 0{\cdot}05$) after ketamine HCl and sodium pentobarbital. The depression in SCC (O - - - O) is significant at 30 min ($P < 0{\cdot}001$) and at 2 hr ($P < 0{\cdot}05$) after ketamine HCl but only at 2 hr ($P < 0{\cdot}05$) after sodium pentobarbital. ADCC and SCC activity did not change significantly after meperidine HCl or in the untreated controls.

ADCC and SCC effector activity were tested at several effector/target cell ratios and 50% LU calculated from these data. Table 1 shows ADCC and SCC LU declining significantly only in the groups given ketamine HCl and sodium pentobarbital. In agreement with the data calculated at a fixed E/T ratio, the lowest point of ADCC LU was at 30 min, with gradual recovery thereafter. At 48 hr, however, the mean LU of ADCC effector activity was still depressed in both the ketamine HCl and sodium pentobarbital groups.

Since ADCC and SCC functions are mediated principally by nonsensitized lymphocytes, we sought to determine whether the cytolytic effector function of antigen-specific, sensitized lymphocytes was also susceptible to inhibition by intravenous anaesthetic agents (Fig. 2). In these experiments ketamine HCl caused a mean 80% ($P < 0{\cdot}001$) decline in cytotoxic T lymphocyte effector activity within 30 min; a mean 67% decline ($P < 0{\cdot}025$) occurred after sodium pentobarbital. Meperidine HCl did not depress effector activity.

These data show that administration of a single, fixed intravenous dose of ketamine HCl or sodium pentobarbital is associated with a rapid, statistically significant reduction in diverse cytolytic effector cell functions; meperidine HCl has no such effect. The changes represent a major depression in the efferent limb of host cellular immune defense mechanisms.

Changes in lymphocyte proliferative response to mitogens

To determine whether ketamine HCl, sodium pentobarbital, and meperidine HCl altered mitogen-induced proliferative capabilities of Rhesus lymphocytes, PBMC were assayed before and serially after administration of these agents. Con A responses in the ketamine HCl and control groups did not change significantly (Fig. 3a). In animals given meperidine HCl and sodium pentobarbital, however, a significant decline in the Con A response occurred at 2 hr. For the monkeys given meperidine HCl, this represented a 92% reduction in mean c.p.m. ($P < 0{\cdot}001$); the group given sodium pentobarbital showed 50% reduction ($P < 0{\cdot}02$).

Table 1. Changes in ADCC and SCC lytic units after anaesthesia

Anaesthetic agent	Hours after anaesthesia	Mean lytic units 10^7 effector cells ± s.d.* ADCC	SCC
Ketamine HCl	0	43·6 ± 13·3	13·2 ± 2·6
	0·5	15·1 ± 8·2‡	5·0 ± 2·3‡
	2	23·0 ± 10·8†	7·9 ± 4·0†
	48	28·8 ± 8·8	8·4 ± 5·2
Sodium pentobarbital	0	60·1 ± 10·9	16·3 ± 1·4
	0·5	12·5 ± 1·9‡	13·5 ± 4·8
	2	22·0 ± 10·6†	6·6 ± 2·5‡
	48	36·6 ± 9·8	18·1 ± 10·1
Meperidine HCl	0	47·3 ± 17·6	15·2 ± 6·8
	0·5	41·5 ± 14·9	17·4 ± 8·9
	2	33·5 ± 8·7	12·2 ± 3·5
	48	49·6 ± 1·6	15·0 ± 4·7
Control	0	39·6 ± 6·7	19·7 ± 10·5
	0·5	36·6 ± 15·4	18·1 ± 13·5
	2	35·0 ± 12·1	16·7 ± 7·6
	48	35·2 ± 2·8	18·2 ± 6·4

* A lytic unit was defined as the number of effector cells required to lyse 50% of the target cell suspension. Each value represents the mean obtained from 5 animals.

† $P < 0.05$ compared to the pre-treatment value by two-tailed Student's *t*-test.

‡ $P < 0.01$.

Lymphocyte responsiveness to staphylococcal protein A became significantly depressed only in the group given meperidine HCl (Fig. 3b). The magnitude of this reduction in proliferative response was clearly less, however, than that observed for Con A.

Changes in WBC and mononuclear cell subpopulations

WBC and lymphocyte counts fluctuated only minimally, with no direct relationship to changes in cytolytic effector activity or lymphocyte proliferative activity described above.

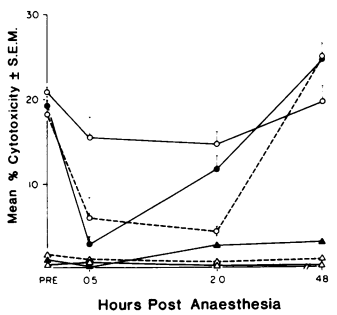

Fig. 2. Changes in cytotoxic activity of allospecific sensitized T cells after *in vivo* exposure to intravenous anaesthetic agents. Results are expressed as the mean percent lysis ± s.e.m. obtained after a 4 hr incubation period, using an effector to target cell ratio of 75:1. Target cells were chromium-51 tagged specific allogenic donor lymphocytes (●, ○) and autologous lymphocytes (▲, △). Cytotoxic activity is compared after ketamine HCl (●——●), sodium pentobarbital (○ - - ○) and meperidine HCl (○——○).

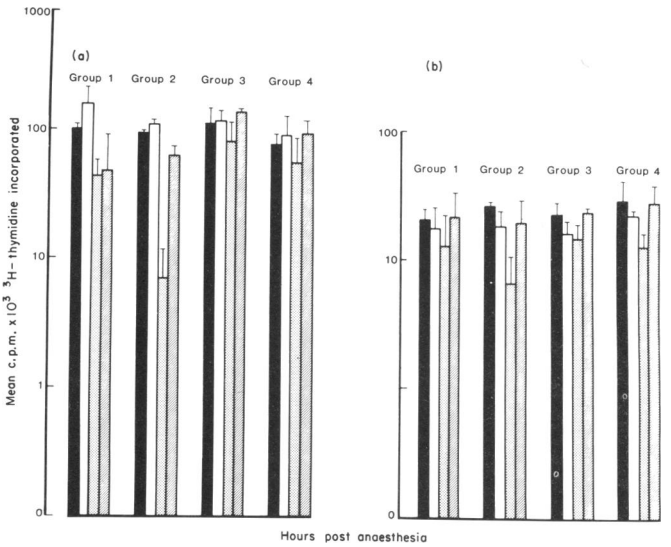

Fig. 3. (a) Changes in concanavalin A-induced lymphocyte transformation after *in vivo* exposure to sodium pentobarbital (Group 1), meperidine HCl (Group 2), and ketamine HCl (Group 3). PBMC were sampled before anaesthesia (■), 0·5 hr (□), 2 hr (▦), and at 48 hr (▨) after anaesthesia. Controls (Group 4) were untreated. PBMC were cultured with Con A (180 μg/ml) for 72 hr. Results are expressed as mean c.p.m. ^{3}H-thymidine uptake. Significant reduction in ^{3}H-thymidine uptake occurred at 2 hr after meperidine HCl ($P < 0{\cdot}001$) and sodium pentobarbital ($P < 0{\cdot}02$). (b) Changes in staphylococcal protein A-induced lymphocyte transformation after *in vivo* exposure to anaesthetic agents. PBMC were cultured with staphylococcal protein A at a concentration of 200 μg/ml.

To investigate the possibility that diminution in effector cell activity was due to differential loss of lymphocyte subpopulations from the circulation, percentages of PBMC subpopulations were measured. No significant changes appeared in the percentage of T cells or B cells in any group. Thus, the two major lymphocyte subpopulations remained within the normal range in both controls and anaesthetized monkeys.

Fluctuations in percentages of Fc-receptor-bearing lymphocytes were divergent and not obviously associated with changes in effector cell function. Significant elevations occurred in the groups given meperidine HCl ($P < 0{\cdot}005$) and sodium pentobarbital ($P < 0{\cdot}05$). Moreover, peak elevation in Fc receptor cells in these groups coincided with peak depression in Con-A-induced lymphocyte proliferative responsiveness, suggesting a possible relationship between these events.

In vitro *effects of ketamine HCl and sodium pentobarbital*

To determine whether ketamine HCl and sodium pentobarbital impaired effector function of lymphocytes by direct action on the cells, these agents were added *in vitro*. Since reduction in effector activities was optimal within 30 min after *in vivo* administration, we studied the effect of *in vitro* exposure to graded doses of ketamine HCl and sodium pentobarbital after a 30-min incubation period. Data in Fig. 4a show no significant change in ADCC or SCC effector activity after ketamine HCl. Sodium pentobarbital caused no significant changes of ADCC or SCC effector activity at concentrations from 0·0039 to 0·39 mg/ml (Fig. 4). At 3·9 mg/ml, sodium pentobarbital completely inhibited effector activity, which might be explained by the toxic effect on the cells (there was mean 85% loss of cell viability). Within a pharmacological dose range, however, neither ketamine HCl nor sodium pentobarbital inhibited effector functions after *in vitro* exposure. The contrast between *in vivo* and *in vitro* effects of ketamine HCl and sodium pentobarbital suggests an indirect suppressive effect on effector function.

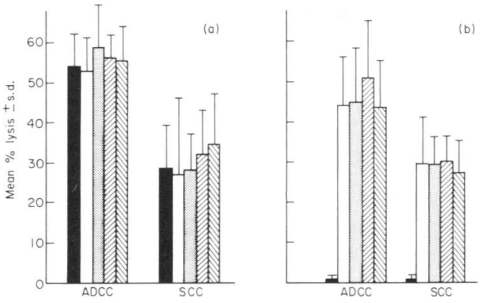

Fig. 4. (a) Effect of *in vitro* exposure of PBMC to ketamine HCl on ADCC and SCC effector function. Ketamine HCl was added to heparinized blood at 1·3 mg/ml ■, 0·13 mg/ml □, 0·013 mg/ml ▤, and 0·0013 mg/ml ▨. PBS ▧ was added as a control. After a 30 min incubation period at 37°C, the PBMC were isolated, washed ×3 and assayed for effector activity. (b) Effect of *in vitro* exposure to sodium pentobarbital at a concentration of 3·9 mg/ml blood ■, 0·39 mg/ml □, 0·039 mg/ml ▤, and 0·0039 mg/ml ▨. PBS ▧ was the control.

Changes in PBMC cyclic nucleotide levels after anaesthesia

Other studies have demonstrated that elevated lymphocyte levels of cyclic cAMP coincide with decreased cytolytic activity (Henney, Bourne & Lichtenstein, 1972; Strom, Lundin & Carpenter, 1977). To investigate the possibility that depressed effector activity after *in vivo* ketamine HCl and sodium pentobarbital might be related to altered intracellular cyclic nucleotide levels, we compared cAMP and cGMP levels in PBMC before and serially after *in vivo* administration of meperidine HCl, ketamine HCl, and sodium pentobarbital. For this experiment meperidine HCl, which we had found not to suppress cytolytic effector cell function significantly, was used as a control. Table 2 shows a significant elevation in cAMP levels only after ketamine HCl. Duration of elevation of cAMP levels coincided with duration of reduced cytolytic effector function. The cGMP levels were not significantly altered. Thus these data associate a metabolic change caused by ketamine HCl with specific alteration in lymphocyte function.

Table 2. Changes in PBMC cyclic nucleotide levels after anaesthesia

Anaesthetic agent	Hours after anaesthesia	cAMP pm/10^6 cells	cGMP pm/10^6 cells
Ketamine HCl*	0	1·120±0·02	1·573±0·16
	0·5	1·860±0·05§	1·409±0·15
	2	2·080±0·33‡	1·075±0·49
	48	1·670±0·45	1·186±0·23
Sodium pentobartibal†	0	0·336±0·16	0·739±0·06
	0·5	0·460±0·03	0·817±0·26
	2	0·540±0·01	0·679±0·52
	48	0·346±0·12	0·877±0·14
Meperidine HCl†	0	0·251±0·04	0·582±0·22
	0·5	0·155±0·04	0·515±0·11
	2	0·261±0·08	0·515±0·22
	48	0·264±0·29	0·188±0·05

* Results expressed as mean of 3 experiments ± standard deviation.
† Results expressed as mean of 2 experiments ± standard deviation.
‡ $P<0·05$ compared to pre-treatment value by two-tailed Student's *t*-test.
§ $P<0·005$.

DISCUSSION

Immunosuppressive effects of *in vivo* administered anaesthesia have been the focus of numerous studies. A few reports have challenged the thesis that anaesthetic agents *per se* induce immune alterations, suggesting instead that surgical or trauma-related stress alters lymphocyte function in postoperative patients (Jubert *et al.*, 1973; Kanto, Vapaavuori & Viljanen, 1974). In this study we sought to determine whether anaesthetic agents can induce alterations in the immune system of a higher primate species. Primates, which show defined similarities with humans in the metabolism of anaesthetic agents, were used to closely approximate a human model (Caldwell *et al.*, 1979).

This report constitutes a comprehensive immunologic functional evaluation of three distinct types of intravenous anaesthetic agents. *In vivo* administration of these agents brought about statistically significant reduction in the immunologic functional capabilities of peripheral blood lymphocytes, but their specific effects on the cellular immune system were dissimilar. The data indicate, therefore, a differential sensitivity of monkey lymphocyte subpopulations to inhibition by these intravenous agents.

We offer evidence that a single *in vivo* exposure to ketamine HCl or sodium pentobarbital can significantly depress the cytolytic effector activity of circulating lymphocytes in normal rhesus monkeys. The influence of intravenous anaesthetic agents on immune effector cell function has not previously been studied, although reduction of ADCC and SCC effector cell function has been reported in surgical patients who have undergone inhalation anaesthesia (Kumar & Taylor, 1974; Vose & Moudgil, 1976; Micheels, Degiovanni & Castermans, 1978). Furthermore, *in vitro* exposure of lymphocytes to halothane and nitrous oxide reduced alloantigen specific T cell cytotoxicity (Cullen, Duncan & Ray-Keil, 1976). These findings indicate that cellular immune effector mechanisms in primates and man can be inhibited by various anaesthetic agents.

Studies in human and subprimate species indicate that the effector cells which mediate ADCC, SCC, and antigen-specific cytotoxic T lymphocyte reactions belong to discrete subpopulations of lymphocytes (Weksler *et al.*, 1977). Classification of effector cells has not yet been established for the rhesus monkey. If the current concept of effector cell heterogeneity is assumed for this species, then inhibition of all three effector reactions after ketamine HCl or sodium pentobarbital anaesthesia may be due to interference with a mechanism common to these various cytolytic reactions.

Previous studies have demonstrated an inverse relationship between effector cell cAMP levels and the expression of cytotoxicity (Henney, Bourne & Lichtenstein, 1972; Strom, Lundin & Carpenter, 1977). We showed that cAMP levels were elevated in PBMC after *in vivo* exposure to ketamine HCl. The proximity in time of elevation in lymphocyte cAMP and depression in effector cell activity suggests a relationship between these events.

In contrast to effects seen after *in vivo* exposure to ketamine HCl, *in vitro* exposure of PBMC to ketamine HCl for 30 min did not suppress cytolytic effector function, indicating that inhibition depended upon some intermediary factor. Ketamine HCl is known to be a sympathomimetic agent. Epinephrine can increase cAMP levels and inhibit both cytolytic K and T effector cell function (Strom, Lundin & Carpenter, 1977). It is possible, therefore, that a neurotransmitter substance, perhaps epinephrine, is involved in the inhibition of effector function by ketamine HCl. Absence of inhibition of effector function after *in vitro* exposure to sodium pentobarbital in a pharmacologic dose range indicates that its *in vivo* suppressive action occurs, like that of ketamine HCl, through an indirect mechanism. Inhibition of effector cell activity by sodium pentobarbital was not associated with intracellular changes in cAMP, suggesting a mechanism different from that of ketamine HCl.

Data from the mitogen-induced lymphocyte proliferation assays show effects of anaesthesia distinct from those observed in the cytotoxicity assays. Although ketamine HCl significantly impaired the cytolytic function of effector lymphocytes, it caused no detectable changes in mitogen-induced lymphocyte proliferative function. Our finding that ketamine HCl does not suppress lymphocyte transformation agrees with Wilson *et al.* (1973), who showed no significant changes in mitogen-induced lymphocyte transformation in children given ketamine HCl.

Previous reports have documented depressed mitogen- and antigen-induced lymphocyte transformation in both surgical patients and laboratory animals after variously induced anaesthesia

(Park *et al.*, 1971; Berenbaum, Fluck & Hurst, 1973; Espanol, Todd & Soothill, 1974; Cooper, Irvine & Turnbull, 1974; Formeister *et al.*, 1980). We observed a moderate reduction in lymphocyte proliferative function in monkeys given sodium pentobarbital, but a striking depression in lymphocyte proliferative responses to both Con A and staphyloccocal A occurred after meperidine HCl. These observations indicate differential sensitivity of lymphocyte proliferative function to inhibition by meperidine HCl, which is in sharp contrast to the absence of inhibition of lymphocyte cytolytic effector function by meperidine HCl.

Reduction in mitogen-induced lymphocyte proliferation after meperidine HCl and sodium pentobarbital occurred within 2 hr, coincident with significant elevation in the percentage of circulating Fc receptor cells. This might possibly implicate a 'traffic shift' in the PBMC subpopulations. Studies of effects of glucocorticoids have demonstrated that transient depletion of PBMC subpopulations can cause enrichment of cells resistant to effects of glucocorticoids (Fauci, 1979). In humans, suppressor T cells, which bear Fc receptors for IgG, are resistant to lymphodepletive effects of steroids (Fauci, 1979). Transient imbalance in suppressor and helper T cell subsets can produce net reduction of lymphocyte reactivity; such a mechanism might be involved in the immunosuppressive action of sodium pentobarbital and meperidine HCl on lymphocyte proliferative function.

Lymphocyte proliferative responses and cytolytic effector cell functions are believed to play a major *in vivo* role in host defense against infection and tumours (Kasai *et al.*, 1979; McCredie, 1980; Penn, 1979; Roder *et al.*, 1980; Weksler *et al.*, 1977). Our study shows that a minimal anaesthetic dose can induce significant alteration in either afferent or efferent cellular immune function. Since suppression of cellular immune function in anaesthetized normal primates persisted for at least 2 hr, prolonged exposure to anaesthesia, increased doses of anaesthetic agents, or both will likely exacerbate these immunosuppressive effects. Thus, when the immune system is already depressed by drug therapy, malignancy, or malnutrition, prolonged anaesthesia may aggravate the clinical condition.

We are grateful to Dr Bob Balster for stimulating discussions of the data, to Mr Willie Boone and Ms Susan Juoska for skilled technical assistance, and to Mrs Betsy White for preparation of the manuscript.

This work was supported by Grant No. RO-AI 16930 of the National Institutes of Health.

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