which are connected with each other by internodal strands. Plenty of bright fluorescent adrenergic axons could be localized. A network of varicose nerve fibers is seen all over the ganglia and the internodal strands (fig. 1). The intramural ganglion cell bodies could be observed in between the varicose fibers as oval black patches. The action of 5,7-DHT is as follows;

24 h after treatment a few nerve fibers disappear and some of the persisting fibers become swollen. However, more than 50% of the fibers remain unaffected (fig. 2). 48 h after treatment a few more adrenergic nerve fibers disappear. 10 days after 5,7-DHT administration almost all the lost fibers reappear and the stretch preparations look almost similar to the control ones.

On comparing the action of 5,7-DHT with 6-OHDA it was noted that 6-OHDA could suppress the fluorescence of more adrenergic profiles than 5,7-DHT.

The findings of the present study suggest that treatment with 5,7-DHT was responsible for only partial disppearance of adrenergic nerve profiles, as more than 50% of the total population of the nerve terminals remained unaffected. It has also been shown that 6-OHDA is more potent than 5,7-DHT for chemical sympathectomy. The swelling of nerve fibers after 5,7-DHT and 6-OHDA might result from a building up of catecholamine within the preterminals, which is suggestive of block of axoplasmic flow of noradrenaline within the nerve trunks probably due to damage of the more peripheral terminal varicosities[3,4,7]. Intensely fluorescent preterminals with swollen and distorted bodies were induced by 6-OHDA treatment in Auerbach's plexus of ileum[8], colon[5] and oviduct[6], and in the atria, submaxillary glands and irides[9]. The accumulation of catecholamine within sympathetic neurons after axonal section or constriction has been reported[10]. The reappearance of adrenergic nerve terminals 10 days after 5,7-DHT treatment possibly demonstrated the process of regeneration. During the process of regeneration it appears that nerves regain the ability to take up norepinephrine more rapidly than their ability to store, retain or synthesize norepinephrine[11,12].

1 The author is thankful to Professor G. Burnstock, Department of Anatomy and Embryology, University College London, London, England (where part of the work was done) for providing facilities.

2 Laverty, R., Sharman, D.E., and Vogt, M., J. Pharmac. *24* (1965) 549.
3 Malfors, T., and Sachs, Ch., Br. J. Pharmac. *3* (1968) 89.
4 Thoenen, H., and Tranzer, J.P., Naunyn-Schmiedebergs Arch. Pharmak. *261* (1968) 271.
5 Qayyum, M.A., and Fatani, J.A., Zool. Anz. Jena *210* (1983) 91.
6 Qayyum, M.A., Fatani, J.A., and Maleika, S.S., J. Sci. Res. *5* (1982) 1.
7 Tranzer, J.P., and Thoenen, H., Naunyn Schmiedebergs Arch. Pharmak. *257* (1967) 343.
8 Qayyum, M.A., Acta anat. *96* (1976) 497.
9 Goldman, H., and Jacobowitz, D., Pharmac. exp. Ther. *176* (1971) 119.
10 Dahlstrom, A., and Fuxe, K., Acta physiol. scand. *64* (1965) 1.
11 Johnsson, G., and Sachs, Ch., Eur. J. Pharmac. *9* (1970) 14
12 Johnsson, G., and Sachs, Ch., J. Pharmac. exp. Ther. *180* (1972) 625.

0014-4754/83/121385-02$1.50 + 0.20/0

Effect of general anesthetics on human granulocyte chemiluminescence

S. Lippa, P. De Sole, E. Meucci, G.P. Littarru, G. De Francisci and S.I. Magalini

Institute of Biological Chemistry, and Institute of Anaesthesiology, Università Cattolica del Sacro Cuore, Via Pineta Sacchetti 644, I-00168 Rome (Italy), November 17, 1982

Summary. The effect of general anesthetics on human granulocyte 'phagocytic capacity' was tested, both in vivo and in vitro, by means of chemiluminescence. Halothane and ethrane produced a consistent degree of chemiluminescence inhibition, which, in vitro, was clearly dose-dependent.

There is increasing evidence that exposure to anesthesia and surgery has important immunological implications[1]. A great effort has been made to assess the possible effect of general anesthesia on both specific immunity and nonspecific resistance mechanisms. Within the non-specific resistance mechanims the key role of phagocytosis is well established. Several studies have demonstrated the adverse effect of general anesthetics on the various steps of phagocytosis by polymorphonuclear leukocytes (PMN-L), such as mobilization[2] chemotaxis[3,4] transvascular diapedesis[5] and the final bactericidal events[6].

The aim of this paper is to investigate the degree of 'phagocytic capacity and related metabolic activation' in circulating PMN-L obtained from surgical patients under general anesthesia: this investigation and that of the in vitro effect of halothane and of ethrane on a purified suspension of PMN-L have been performed by means of chemiluminescence (CL).

It is now widely recognized a good correlation between PMN-L chemiluminescence and the 'respiratory burst'; indeed the oxidative microbicidal activity of PMN-L is associated with the generation of electronically excited product molecules: the relaxation to the ground state by photon emission results in the phenomenon of CL[7]. The use of high quantum yield molecules, such as luminol (5-amino-2,3, dihydro-1, 4-phtalazinedione), leads to an amplified CL response.

Materials and methods. Patients selected for this study underwent minor surgery operations. Peripheral venous blood was collected, in heparinized tubes (heparin: lithium salt) before general anesthesia (GA) and 1 h after the inducement of GA. Patients were anesthetized with ethrane (concentration: 1.5%) or with halothane (concentration 1%). The patients received no blood transfusion during the 1 h period of general anesthesia. PMN-L were separated from whole blood by the Ficoll-hypaque density gradient centrifugation followed by dextran sedimentation and 30-sec hypotonic lysis with 0.23% NaCl[8].

Isolated PMN-L were suspended in a modified Krebs Ringer phosphate (KRP) medium, pH 7.4 (without $CaCl_2$

and with the addition of 0.33 mM Ca $(NO_3)_2 \cdot 4\ H_2O$ and 5 mM glucose). Chemiluminescence was measured with a Packard Beta Scintillation counter, at the tritium setting, in the coincidence mode. Each plastic scintillation vial contained, in a 5-ml KRP final volume, 2×10^6 PMN-L, and 100 µl of a 50 µM solution of luminol (Sigma) in dimethyl sulfoxide. Phagocytosis was started by the addition of 0.66 mg of latex beads (diameter: 0.1 µm; Serva Feinbiochemia).

For the in vitro experiments, PMN-L were obtained from normal blood donors by the separation technique mentioned above. Aliquots of a PMN-L suspension (10^6 cells/ml) were exposed for 10 min to halothane or to ethrane, in air, at the concentrations indicated in the results section.

The anesthetic-air mixture was bubbled into the leukocyte suspension with a flux of 60 ml/min. Blanks were treated with air only (60 ml/min).

Results. Table 1 shows the CL response of PMN-L from a group of patients under general anesthesia (blood was usually drawn 60 min after the onset of general anesthesia). As can be seen in the right column, in the cases where halothane was used CL was always depressed, the degree of inhibition varying from 39 to 89% with respect to the pre-anesthesia values. In the cases where ethrane was used results were less uniform; inhibition was found in 5 out of 8 cases, and was nearly complete in 2 of these patients. But in the remaining 3 patients no inhibition occurred, 1 of these patients being stimulated instead.

Figure 1 shows the in vitro effect of halothane on PMN-L chemiluminescence. Data are referred to PMN-L from 3 different normal subjects. After a 10-min exposure to halothane inhibition was clear even at the lowest concentration of the anesthetic (0.3%). By increasing the halothane percentage towards 1.1% the extent of inhibition rose to values ranging from 75% to 100% with respect to the basal value. Figure 2 shows the in vitro effect of ethrane on PMN-L chemiluminescence. The inhibition is comparable to or even more pronounced than that just seen for halothane.

Discussion. The rather low final amount of luminol we adopted was selected from a dose-response curve previously determined[9], since it allows a reasonable sensitivity together with a good precision. The use of latex beads as a stimulating agent was preferred to the use of zymosan, in order to avoid the introduction of another variable, i.e., the opsonization.

Effect of general anesthesia on human PMN-L chemiluminescence

Anesthetic	PMN-L stimulated CL (cpm × 10^{-3}) Pre-anesthesia	During anesthesia	Inhibition (%)
Halothane	240	87	76
Halothane	240	118	51
Halothane	710	390	45
Halothane	990	105	89
Halothane	185	92	51
Halothane	860	280	68
Halothane	310	109	39
Ethrane	15	0.6	96
Ethrane	850	540	37
Ethrane	600	840	No inhibition
Ethrane	620	450	28
Ethrane	50	0.4	99
Ethrane	575	310	46
Ethrane	1550	1700	No inhibition
Ethrane	615	1100	80% stimulation

Chemiluminescence (CL) was determined on isolated polymorphonuclearleukocytes (PMN-L) from surgical patients, before general anesthesia (GA) and 1 h after the inducement of GA. Latex beads (diameter: 0.1 µm) were used as phagocytic stimulus. CL values are expressed as cpm readings at the plateau.

The remarkable inhibitory effect that both halothane and ethrane show in vitro on PMN-L chemiluminescence might find a reasonable biochemical explanation in the primary labilization of lipid-protein interactions that general anesthetics produce on biological membranes[10]. It must be stressed that in our experiments, at the time of CL measurements neutrophils were not in contact any more with the anesthetics. We should therefore conclude that contact with even small concentrations of anesthetics for short periods of time results in damage to PMN-L membranes that lasts some time.

Since the medium was buffered it is unlikely that there was any major modification of its pH by GA. In any case, previous experiments specifically designed to assess the

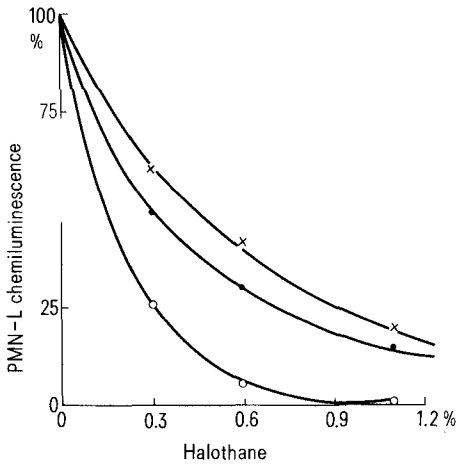

Figure 1. In vitro effect of halothane on PMN-L chemiluminescence. Purified PMN-L suspensions (10^6 cells/ml) were exposed for 10 min to different percentages of halothane (in air). Chemiluminescence was recorded upon inducement of phagocytosis with latex beads (diameter: 0.1 µm). CL values are expressed as CPM readings at the plateau. The different curves refer to PMN-L from 3 different normal subjects. Each value is the mean of 3 different determinations.

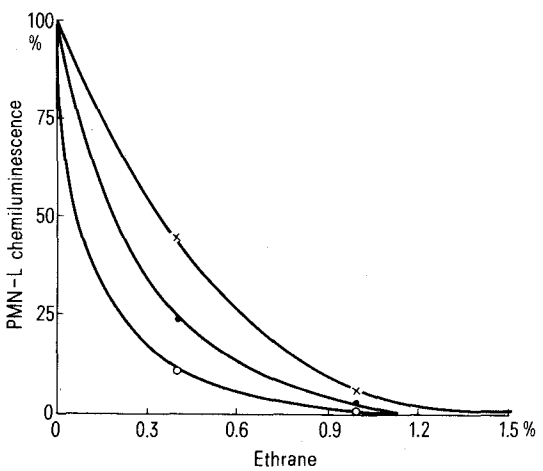

Figure 2. In vitro effect of ethrane on PMN-L chemiluminescence. Purified PMN-L suspensions (10^6 cells/ml) were exposed for 10 min to different percentages of ethrane (in air). Chemiluminescence was recorded upon inducement of phagocytosis with latex beads (diameter: 0.1 µm). CL values are expressed as CPM readings at the plateau. The different curves refer to PMN-L from 3 different normal subjects. Each value is the mean of 3 different determinations.

effect of pH variations on PMN-L chemiluminescence, showed no significant change of CL response in the range of pH from 6 to 8 (unreported data). As could be expected, the in vivo experiments resulted in a less uniform behavior of the CL response, although the inhibitory trend was the rule for the patients who underwent anesthesia by halothane, and inhibition was also the most common response for the cases where ethrane was used. Since GA were shown in vitro to inhibit CL, the inhibition observed in vivo is therefore likely to depend on anesthesia in the first place. Here again we must notice that, because of the time needed for blood transportation and PMN-L separation from whole blood, chemiluminescence was measured $2\frac{1}{2}$ h after the in vivo exposure of PMN-L to halothane or to ethrane.
The lack of uniformity in the extent of inhibition, mainly within the ethrane group, might reasonably be supposed to be due to a partial reversal of the inhibitory effect of GA on chemiluminescence. According to our data, the inhibition of chemiluminescence by GA is more pronounced than the inhibition of phagocytosis by GA, which other authors have shown by means of closely related techniques, i.e. latex ingestion and NBT reduction[11]. Our results on human PMN-L are in agreement with the anesthetic depression of rat lung macrophages that Graham et al.[12] demonstrated, also by a chemiluminescence technique.
An in vivo study designed to assess the time necessary for the restoration of a normal chemiluminescence response after inducement of general anesthesia is currently under way.

1 Walton, B., Br. J. Anaesth. *51* (1979) 37.
2 Bruce, D.L., and Wingard, O.W., Anaesthesiology *34* (1971) 271.
3 Moudgil, G.C., Allan, R.B., Russell, R.J., and Wilkinson, P.C., Br. J. Anaesth. *49* (1977) 97.
4 Hill, G.E., Stanley, T.H., Lumn, J.K., Liu, W.S., English, J.B., Loeser, E.A., Kawamura, R.K., Seutker, C.R., and Hill, H.R., Anaesth. Analg. *56* (1977) 696.
5 Magalini, S.I., and Scrascia, E., IVth Eur. Congr. Anaesth., Madrid; abstracts. Excerpta Medica, Amsterdam 1974, p. 141.
6 Manawadu, B.R., and LaForce, F.M., Chest *75* (1979) suppl. 242.
7 Allen, R.C., in: Liquid scintillation counting. Recent applications and development, volume II. Sample preparation and applications, p. 377. Eds P.E. Stanley and B.A. Scoggins. Academic Press, New York 1980.
8 Castelli, A., De Sole, P., Gozzo, M.L., Littarru, G.P., and Martorana, G.E., Ital. J. Biochem. *22* (1973) 4.
9 De Sole, P., Lippa, S., Rotilio, G., and Finazzi Agrò, A., Int. Meeting Inflammation, Verona 1979; abstracts, p. 76.
10 Lenaz, G., Curatola, G., Mazzanti, L., and Bertoli, E., Ital. J. Biochem. *30* (1981) 290.
11 Cullen, B.F., Hume, R.B., and Chretien, P.B., Anaesth. Analg. *54* (1975) 501.
12 Graham, C.W., Welch, W.A., and Zaccari, J., Anaesthesiology *51* (1979) 548.

0014-4754/83/121386-03$1.50 + 0.20/0

Inhibitory effect of α-α-diphenyl-α-propoxyacetic acid-L-methyl-4-piperidyl ester hydrochloride on the activity of the rat urinary bladder

M. Nagai, M. Nakajima, S. Usuda and M. Iriki

Department of Physiology, Medical University of Yamanashi, Tamaho, Nakakoma, 409-38 Yamanashi (Japan), May 25, 1983

Summary. α-α- Diphenyl-α- propoxyacetic acid-l-methyl-4-piperidyl ester hydrochloride(propiverine) significantly decreased the volume-pressure ratio of the rat urinary bladder and suppressed efferent nervous activity of the bladder branch of the pelvic nerve during vesical extension.

α-α-Diphenyl-α-propoxyacetic acid-l-methyl-4-piperidyl ester hydrochloride(propiverine) is a derivative of benzyl acid, and has been reported to exhibit spasmolytic and analgesic effects[1]. A parasympathicolytic effect of propiverine has been suspected, while propiverine attenuated both the acetylcholin- and nicotine-spasm of the isolated ileum of the guinea-pig. In the present experiments, effects of propiverine on the volume-pressure curve of the urinary bladder and pelvic nervous activity were investigated in the rat. Since activation of the detrusor muscle of the rat urinary bladder is due to acetylcholine release from the pelvic nerve endings[2,3], investigation of urine excretion of the rat can be used to investigate the effect of a drug on the parasympathetic effector system.
Male albino rats of the Sprague-Dawley strain, weighing 300–540 g, were used. Animals were anesthetized with urethane, 1 g/kg i.p., before the surgical operation, and a supplemental dose of 150–180 mg of urethane was injected i.v. during experiments, if necessary. The urinary bladder was exposed following midline-incision, and a polyethylene cannula was inserted from the apex. This cannula was connected with a pressure transducer, and simultaneously with an infusion pump by bifurcation of the transducer head. Intravesical pressure was varied by infusing saline through the cannula at a constant rate by means of the infusion pump. Mass discharges of the efferent pelvic nerves (PNA) were recorded with bipolar Pt-electrodes distal to the pelvic plexus. Afferent nervous activity of the pelvic nerve was eliminated by pinching the nerve bundle distal to the recording electrodes. The hypogastric ganglion was ligatured beforehand, in order to avoid sympathetic influence descending through hypogastric nerves. Efferent pelvic nervous activity (PNA) was evaluated semiquantitatively by integrating mass discharges over a 1-sec period. Body temperature of the animal was kept constant, not lower than 36 °C, during experiments, adjusting heat pad

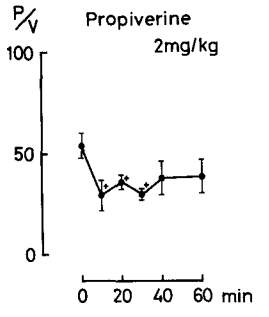

Figure 1. Effect of propiverine on volume-pressure ratio (P/V) of the rat urinary bladder. Means and SE for 4 animals. Propiverine, 2 mg/kg i.v.+, data significantly different from those of control (Student's t-test, $p < 0.05$).