The effect of halothane on in vitro human neutrophil chemotaxis

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Summary. Increasing concentrations of halothane were shown to sequentially inhibit directed (chemotactic) and random movement of human peripheral blood neutrophils. No influence on neutrophil movement was apparent at clinically important concentrations, halothane may effect neutrophil microtubule and actomyosin microfilament systems.

The exact mechanism by which neutrophils and other phagocytes move about remains incompletely understood. However, increasing evidence both ultrastructural and biochemical suggests that a class of organelles, the microtubules may be important for the mobility of phagocytes[3-5]. These tubular structures, about 25 nm in diameter and 5 nm in wall thickness, are high molecular weight protein polymers which can exist in dynamic equilibrium with subunits of their constituent protein, tubulin[6]. Prominent in the peripheral cytoplasm of phagocytes are other structures composed of fine filaments, 4–7 nm in diameter, with contractile and biochemical properties of actomyosin systems[7-10]. It has been postulated that general or random locomotion is provided by the actomyosin contractile system and that orderly directed movement (chemotaxis) depends on microtubule function probably in coordination with actomyosin filaments[4,8,10,11]. A variety of chemical agents including colchicine[12,13], vincristine[14] and griseofulvin[15] can impede neutrophil chemotaxis presumably by interfering with microtubule assembly. In the case of colchicine, and probably the vinca alkaloids, interference results from drug binding with tubulin subunits and a consequent shift in the equilibrium between tubulin monomers and functional microtubule polymer[4,6,16].

It has recently been demonstrated that halothane is capable of reversibly dispersing microtubules in heliozoon organisms[17,18] and immobilizing human lymphocytes[19]. In spite of the apparent effects of halothane on the microtubule systems of a variety of cell types, no influence on the phagocytic properties of human neutrophils has been observed[20]. This is not unexpected since phagocytosis appears to depend primarily on a functioning actomyosin microfilament system and is independent of microtubule function[4]. The observed effects of halothane on microtubule formation suggested that neutrophil movement, especially directed (chemotactic), could be modified by exposure to halothane.

Utilizing a Boyden chamber method[21,22] the effects of various concentrations of halothane on random and directed movement of human polymorphonuclear leukocytes were studied. Peripheral neutrophils were harvested from healthy adult donors by dextran sedimentation and centrifugation and resuspended in TC Medium 199 (Gibco). The cell suspensions were standardized to 5×10^6 leukocytes per chamber. The lower compartment contained either TC Medium 199 – 10% pooled human serum (for measurement of random movement) or TC Medium 199 – 10% human serum with 2 mg/ml caseinate as a chemotaxin. A single filter technique with a 5 μm Sartorius filter was used. Chemotaxis chambers were sealed in containers through which either air or halothane-air mixtures were passed at identical flow rates. All experiments were conducted at 37 °C with an incubation of 2.5 h. Preliminary experiments indicated that complete equilibration of halothane with cell suspensions was accomplished in less than 10 min. Filters were processed for microscopy and neutrophils on the bottom surface of each filter were counted in 5 random high power ($\times$ 400) fields. The result of each experimental condition was the average of three chambers. The effect of halothane on either random or directed movement was expressed as a percentage of the appropriate air control. No attempt was made to examine possible reversibility of halothane effect.

Except at extremely high concentrations (> 20%), halothane did not appear to effect random neutrophil movement. However, directed movement was significantly impaired in the presence of 10% halothane ($p < 0.01$, Student's t-test). At lower concentrations there was no effect on either random or directed movement.

Halothane concentration %	Number of migrated cells; halothane treated cells as % of air controls (mean ± S.D.) Random movement	Directed movement	No. of cell populations
0.5	94 ± 25	120 ± 25	12
2	110 ± 30	89 ± 10	12
4	120 ± 32	139 ± 24	8
10	91 ± 6	15 ± 2	8
20	0	0	6
33	0	0	8

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Interference with directed movement in the absence of a coincident effect on random mobility as seen at 10% halothane suggests depression of microtubule function without a concurrent effect on the actomyosin microfilament system. At extremely high concentrations (> 20%), halothane probably interferes with both microtubule and microfilament systems resulting in complete paralysis of movement. Although no effect on phagocytosis has been observed at lower concentrations (0.5–2.5%) of halothane[20], total immobilization observed at higher concentrations (> 20%) should be associated with impaired phagocytosis. However, no data are available concerning this possibility.

It has been suggested that the negative inotropic effect of halothane on cardiac muscle is the result of inhibition of ATP utilization by the actomyosin contractile system[23]. A similar effect on neutrophil actomyosin ATP utilization could explain the immobilization observed with high concentrations of halothane. It is concluded that clinically significant concentrations of halothane do not effect neutrophil movement in vitro. Higher concentrations sequentially effect chemotactic and random movement.

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The local anesthetic potency of norcocaine, a metabolite of cocaine

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Summary. The local anesthetic effects of cocaine and one of its main metabolites norcocaine, were investigated comparatively on isolated ganglion cells of the marine gastropod, *Aplysia californica*. During a 1-hour-period, different action potential parameters such as amplitude, duration, maximum rate of rise were observed, which demonstrated that norcocaine exhibits a higher local anesthetic potency than cocaine.

Previous experiments have demonstrated a rapid onset of cocaine *N*-demethylation after cocaine is administered in vivo, suggesting the formation of norcocaine as a cocaine metabolite[2]. Recently norcocaine was found to be present in plasma, brain and cerebrospinal fluid of monkeys in considerable concentrations after intraperitoneal cocaine injection[3,4]. In previous reports, the pharmacological activity of cocaine and norcocaine was examined comparatively. Norcocaine inhibited the cardiac response to tyramine more actively than cocaine[4] and both drugs inhibited the uptake of ^{3}H-noradrenaline by rat brain synaptosomes similarly[3]. On the frog sciatic nerve, norcocaine seemed to cause more local anesthetic activity than cocaine[4]. For a detailed comparative analysis of the local anesthetic potencies of cocaine and norcocaine, the ganglion cells of a mollusc, *Aplysia californica*, were used for the experiments. This preparation permits intracellular recording for periods of hours allowing both compounds to be tested on the same neuron under controlled conditions.

Materials and methods. The dissected visceral ganglion of the marine gastropod was placed into a perfusion chamber. Cells identified according to the nomenclature of FRAZIER et al.[5] were penetrated by micropipettes which were filled with 2 *M* potassium citrate having an electrical resistance of 8–15 MΩ. Standard intracellular recording techniques were used.

Artificial seawater (pH = 8.2) was used as perfusate containing cocaine and norcocaine in a concentration of 10 m*M*. This concentration is lower than the effective dosage for local anesthesia[6,7]; however, it was sufficiently effective to reduce inward current dependent voltage changes. The substances were examined in 7 experiments. In each experiment the effect of cocaine and norcocaine was studied on the same cell. Between each drug application, the ganglion was rinsed for at least 2 hours with artificial sea water, until the action potential parameters recovered to at least 90% of the control values measured at the beginning of the experiment. Furthermore, the sequence of drug examination was changed in each subsequent experiment to eliminate effects dependent upon the sequence of drug administration. Drug applications lasted for 60 min and action potential records were made at 30 and 60 min. Both a conventional display of the action potential (V/t) and a phase plane, i.e. the display of voltage against the first derivative of the action potential (dV/dt) on an X–Y-oscilloscope, were recorded. Cocaine HCl was purchased from E. Merck (Darmstadt,

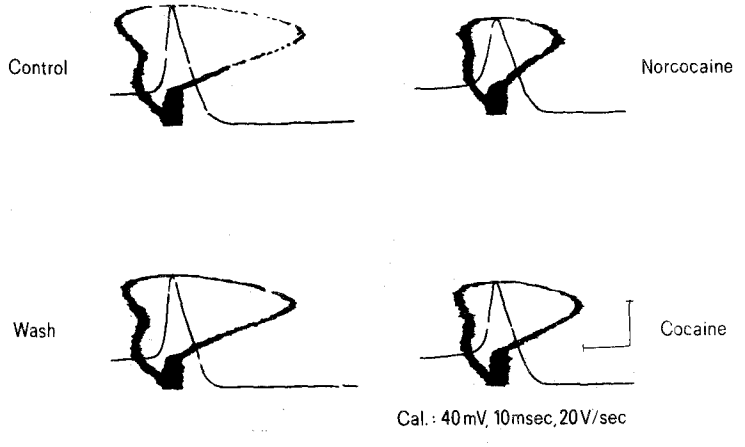

Fig. 1. Influence of a 10 m*M* concentration of cocaine and norcocaine on the action potential and the phase plane of the ganglion cell R-2 of the marine gastropod *Aplysia californica* after 1 h drug contact. Norcocaine revealed a higher local anesthetic potency than cocaine. Note the almost complete recovery of the control action potential after a 2-h-period of washing.