Harbor, NY, 1984), vol. I, pp. 209–260; E. Keshet and H. Temin, *J. Virol.* **31**, 376 (1979); J. I. Mullins, C. S. Chen, E. A. Hoover, *Nature* **319**, 333 (1986); C. D. Pauza, J. E. Galindo, D. D. Richman, *J. Exp. Med.* **172**, 1035 (1990); H. L. Robinson and D. M. Zinkus, *J. Virol.* **64**, 4836 (1990).
12. All patients from whom PBLs were derived for this study were registered in the Clinical AIDS Unit at the University of Nebraska Medical Center, and only patients not undergoing azidothymidine (AZT) treatment were recruited. CD4 and CD8 cell counts were determined at the time blood was drawn for analysis. Isolation of PBL and depletion of monocytes and macrophages was performed essentially as described (*6*). Analysis and quantitation of PCR-amplified products was as described (*6*). The autoradiographic signal of PCR-amplified products from doubling dilutions of DNA from patient lymphocytes was compared with that of PCR-amplified products from doubling dilutions of DNA extracted from 8E5 cells that contain one noninfectious HIV-1 provirus per cell (*13*). HIV-1 DNA copy number in each patient was quantitated after normalization of the autogradiographic signal of HIV-1–specific products to those generated from PCR amplification of primers specific for α-tubulin on parallel dilutions of 8E5 and patient lymphocyte DNA. After quantitation of proviral DNA copy number, lymphocytes were distributed in multiple (10 to 40) aliquots, each of which contained sufficiently few lymphocytes so that there was not more than one infected cell in each aliquot. Typically for asymptomatic individuals, each aliquot contained 400 to 1000 cells, whereas for AIDS patients each aliquot contained 50 to 400 cells. Total cellular DNA was extracted from each aliquot, mixed with purified carrier salmon sperm DNA (1 μg) and resolved on 0.8% low gelling temperature agarose–tris borate gels at 2.5 V/cm for 10 to 14 hours. After electrophoresis, DNA was visualized by ethidium bromide staining. Gel regions containing DNA of 4 to 15 kb (low molecular weight fraction) and over 15 kb (high molecular weight fraction) were excised. Each gel fragment was melted at 68°C, and DNA was extracted and purified as described elsewhere (*6*). Purified DNA from each high and low molecular weight fraction was pelleted, dried, and resuspended in 10 μl H_2O for analysis by PCR.
13. T. M. Folks *et al.*, *J. Exp. Med.* **164**, 280 (1986).
14. M. Stevenson *et al.*, *J. Virol.* **64**, 2421 (1990b).
15. D. D. Ho, T. Moudgil, M. Alam, *N. Engl. J. Med.* **321**, 1621 (1989).
16. A. T. Panganiban and D. Fiore, *Science* **241**, 1064 (1988); W.-S. Hu and H. M. Temin, *ibid.* **250**, 1227 (1990).
17. M. A. Sommerfelt and R. A. Weiss, *Virology* **176**, 58 (1990); F. T. Steck and H. Rubin, *ibid.* **29**, 642 (1966); A. J. Dorner and J. M. Coffin, *Cell* **45**, 365 (1986).
18. E. L. Reinherz *et al.*, *J. Exp. Med.* **150**, 1472 (1979).
19. M. I. Bukrinsky *et al.*, unpublished data.
20. K. C. Deen *et al.*, *Nature* **331**, 82 (1988).
21. P. R. Mueller and B. Wold, *Science* **246**, 780 (1989).
22. D. P. Grandgenett and S. R. Munn, *Cell* **60**, 3 (1990).
23. P. R. Shank *et al.*, *ibid.* **15**, 1383 (1978).
24. G. M. Shaw *et al.*, *Science* **226**, 1165 (1984); S. Y. Pang *et al.*, *Nature* **343**, 85 (1990).
25. PCR was performed essentially as described (*6*), with the following modifications. PCR was performed in a reaction volume of 25 μl, and each cycle of amplification comprised a 30-s denaturation step (95°C), a 30-s annealing step (56°C), and a 1-min extension step (72°C). In addition, there was one 5-min step at 72°C at the end of each series of cycles to ensure complete extension of amplified DNA. The sequences of all primers used in this study are available from M. Stevenson on request.
26. L. Ratner *et al.*, *Nature* **313**, 277 (1985).
27. R. Padmanabhan *et al.*, *Anal. Biochem.* **170**, 341 (1988).
28. We thank R. Gallo, J. Coffin, G. Tarpley, and J. Giam for helpful comments and discussion; J. Goldsmith, J. Pierson, and R. Lovely for providing clinical samples; R. Axel and R. Sweet for CD4; C. Kuszynski for fluorescence cytometry analysis; G. Pallas and S. Diaz for artwork; and K. Hansen and M. Notley for manuscript preparation. The 8E5 cell line was obtained through the AIDS Research and Reference Reagent Program of the NIH. Supported by grants A124481 and A130386 from the NIH, the United Kingdom AIDS-directed program (E30/376), and funds from the Nebraska Research Initiative (M.S.).

18 March 1991; accepted 20 June 1991

Stereospecific Effects of Inhalational General Anesthetic Optical Isomers on Nerve Ion Channels

N. P. FRANKS AND W. R. LIEB

Although it is generally agreed that general anesthetics ultimately act on neuronal ion channels, there is considerable controversy over whether this occurs by direct binding to protein or secondarily by nonspecific perturbation of lipids. Very pure optical isomers of the inhalational general anesthetic isoflurane exhibited clear stereoselectivity in their effects on particularly sensitive ion channels in identified molluscan central nervous system neurons. At the human median effect dose (ED_{50}) for general anesthesia, the (+)-isomer was about twofold more effective than the (−)-isomer both in eliciting the anesthetic-activated potassium current $I_{K(An)}$ and in inhibiting a current mediated by neuronal nicotinic acetylcholine receptors. For inhibiting the much less sensitive transient potassium current I_A, the (−)-isomer was marginally more potent than the (+)-isomer. Both isomers were equally effective at disrupting lipid bilayers.

THE POSSIBILITY THAT OPTICAL ISOMERS of inhalational general anesthetics might display differential biological activities has generally been discounted because of the traditional view that general anesthetics act by nonspecific perturbation of lipid membranes (*1–3*). However, accumulating evidence (*1, 3*) suggests that these "nonspecific" agents may act by binding directly to particularly sensitive protein targets in the central nervous system (CNS). We have observed stereoselective effects of the optical isomers of the widely used inhalational general anesthetic isoflurane on neuronal ion channels, which are generally accepted as the most likely targets for these agents.

Very recently, extremely pure optical isomers of isoflurane (Fig. 1A) were prepared, and the enantiomers of halothane, enflurane, and isoflurane were resolved with the use of capillary columns (*4*). We found differential effects of the two isomers of isoflurane in activating the anesthetic-activated potassium current $I_{K(An)}$ in a previously described (*5*) anesthetic-sensitive neuron in the right parietal ganglion of *Lymnaea stagnalis* (Fig. 1B). This neuron was impaled with two electrodes and then completely removed from the ganglion (*6*), which facilitated rapid exchange of solutions and ensured that the effects of anesthetic were on the neuron itself rather than on the surrounding nerve network. The data in Fig. 1B show that when the cell was voltage-clamped, the $I_{K(An)}$ current induced by bath application of (+)-isoflurane was about twofold greater than that induced by (−)-isoflurane (*7*). This effect was invariably observed and appeared to be independent of anesthetic concentration in the range of isoflurane partial pressures from 0.006 to 0.031 atm, which corresponds to 0.5 to 2.4 times the human minimum alveolar concentration (MAC) of 0.013 atm (*8*). The ratio I^+/I^- of currents induced by the (+)-isomer and the (−)-isomer, respectively, averaged 2.01 ± 0.04 (mean ± SEM, n = 34). The current induced by the racemic mixture was intermediate between those induced by the individual isomers. The current-voltage (I-V) curves for the current activated by each of the enantiomers at a partial pressure of 0.025 atm are shown in Fig. 1C. These curves demonstrate that the differential effects are essentially independent of membrane potential.

The (+)-isomer of isoflurane is also more effective than the (−)-isomer at inhibiting currents (Fig. 2A) induced by the bath application of 200 nM acetylcholine (ACh). For these experiments, we used isolated identified neurons (*9*) from the right parietal ganglion that were very sensitive to ACh but relatively insensitive to isoflurane alone. The neurons were nonetheless clamped at a potential in the range from −70 to −80 mV (near the K^+ reversal potential), which minimized the effect of anesthetic-activation of $I_{K(An)}$, and a low concentration of ACh was used to reduce desensitization. This ACh-induced current had characteristics of a cur-

Biophysics Section, The Blackett Laboratory, Imperial College of Science, Technology & Medicine, Prince Consort Road, London SW7 2BZ, United Kingdom.

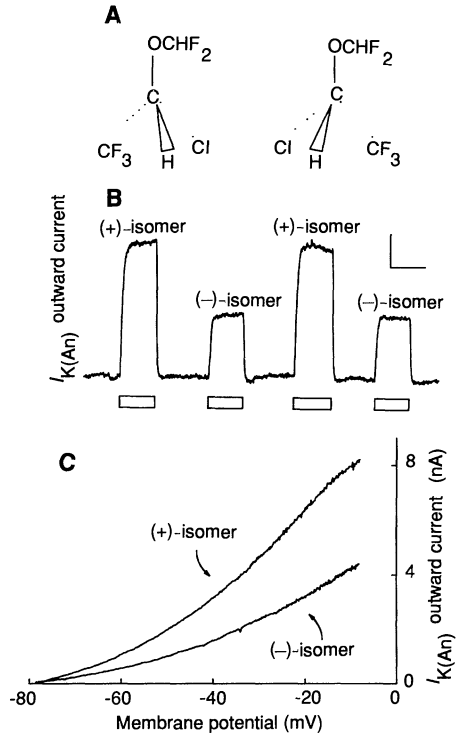

Fig. 1. The (+)-isomer of isoflurane is twice as effective as the (−)-isomer at activating the potassium current $I_{K(An)}$. (**A**) The optical isomers of isoflurane. These were prepared and their purities were analyzed by Anaquest Inc. (Murray Hill, New Jersey), a division of BOC Health Care Inc. Chemical and optical purities were determined by gas chromatography on 60/80 mesh Carbopack B/1% SP-1000 (Supelco) and on chiral Lipodex A (E.S. Industry) columns, respectively. For most experiments, chemical purities were: 99.0% for (+)-isoflurane and 99.1% for (−)-isoflurane; the corresponding optical purity ratios were 99.5%/0.5% and 99.0%/1.0%, respectively. In our early experiments, somewhat less optically pure preparations were used; these gave results consistent with their lower optical purities. The absolute configurations of the two isomers have yet to be established. (**B**) The anesthetic-sensitive neuron from the ventral lobe of the right parietal ganglion (*5*) was isolated as described (*6*). The cell was voltage clamped at 0 mV and bathed in TEA-cobalt saline either with (bars) or without the isoflurane isomers at 0.0065 atm (0.5 MAC). The data shown are a continuous record. The calibration bars represent 0.1 nA and 25 s. (**C**) The differential effects of the isomers in activating $I_{K(An)}$ were independent of voltage. The *I-V* curves are the net currents activated by the isoflurane enantiomers at 0.025 atm and were generated by slowly ramping the membrane potential (at 3 mV s^{-1}) in the depolarizing direction under two-electrode voltage clamp. These data were recorded from an anesthetic-sensitive cell intact in the ganglion, bathed in TEA-cobalt saline. All current records were filtered at 10 Hz. In other experiments the effects of the racemic commercial product Forane (Abbott) in activating $I_{K(An)}$ were found to be indistinguishable from the effects of an equimolar mixture of the two optical isomers.

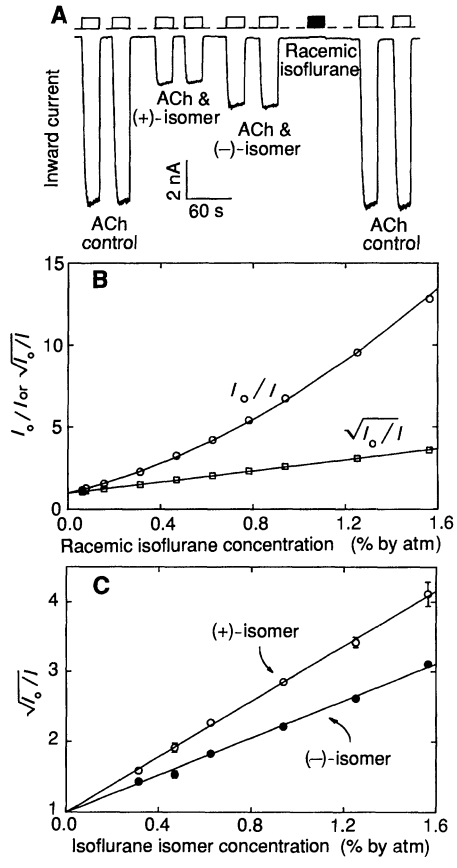

Fig. 2. The (+)-isomer of isoflurane is more effective than the (−)-isomer at inhibiting an inward current evoked by ACh acting at a nicotinic receptor. (**A**) This record shows the effect of repeated applications (open bars) of ACh (200 nM in normal saline) in the presence and absence of 0.005 atm of either the (+)-isomer or (−)-isomer and the lack of an appreciable effect of the racemic mixture (solid bar) on the background holding current in the absence of ACh. The cell was voltage-clamped at −70 mV. The dashed line indicates zero current. Currents were filtered at 10 Hz. (**B**) Two molecules of isoflurane are involved in the inhibition of the ACh-induced current. The ratio (I_o/I) of control to inhibited currents (○), and the square root (□) of this ratio, are plotted as a function of the concentration of racemic isoflurane (Forane), expressed as a partial pressure. The data points are the means of multiple measurements on two separate neurons clamped at −80 mV and exposed to 200 nM ACh. Error bars (± SEM) were smaller than the size of the symbols. The lines are the theoretical predictions of a simple binding model (*11*) in which two molecules of anesthetic can bind independently to the receptor-channel but the presence of only one molecule is sufficient to block it. The mean dissociation constant K_i (here 0.0060 atm for racemic isoflurane) is the reciprocal of the slope of the square root plot. (**C**) The (+)-isomer of isoflurane binds tighter to the ACh receptor-channel than does the (−)-isomer. The square root of the ratio (I_o/I) of control to inhibited currents is plotted against the concentration of (+)-isoflurane (○) and (−)-isoflurane (●). The data points are the pooled means of multiple measurements from nine separate neurons. Error bars (± SEM) are shown when larger than the size of the symbols. The solid straight lines are the predictions of the simple binding model (*11*) for two anesthetic molecules binding with $K_i^+ = 0.0051$ atm for (+)-isoflurane and $K_i^- = 0.0076$ atm for (−)-isoflurane. Note that the straight line for the racemic mixture in (B) would lie between these two lines, with a K_i value almost identical to that predicted (*12*) from the simple binding model (see text).

rent mediated by a neuronal nicotinic receptor (*10*). Two molecules of isoflurane are clearly involved in the inhibition of this current (Fig. 2B), and application of a simple binding model (*11*) gave a mean dissociation constant for racemic isoflurane of 0.0060 atm. The data in Fig. 2C show that each molecule of (+)-isomer binds to the ACh receptor-channel about 50% more tightly than each molecule of the (−)-isomer. [Because of the square dependency of the inhibition, the ratio of currents I^-/I^+ can be considerably larger than that of the binding constants, being 1.71 ± 0.01 (mean ± SEM, n = 20) at the human MAC of 0.013 atm and approaching a limiting value of 2.2 at high concentrations.] The data for the individual isomers (Fig. 2C) predict a mean dissociation constant (*12*) for the racemic mixture of 0.0061 atm, which is almost identical to our observed value (Fig. 2B) of 0.0060 atm.

Both of the neuronal currents discussed above are unusually sensitive to isoflurane, and we were interested in determining if equally large stereoselective effects would be seen with a channel that was much less sensitive to anesthetics. Consequently, we determined the effects of the two isomers on the voltage-gated fast transient potassium current (I_A). At the relatively high concentrations required to inhibit this channel by half (see Fig. 3), the isomers had similar effects, although the (−)-isomer was slightly more potent (ratio of peak currents I^-/I^+ = 0.95 ± 0.01, mean ± SEM, n = 20) at inhibiting the peak outward current. Although this difference is statistically significant, it is small, and it is not easy to rule out some minor contributions due to effects on other ionic conductances.

Our finding that the two very sensitive ion channels show larger chiral effects than the insensitive I_A channel is in line with the general finding of Pfeiffer (*13*) that drugs which act at lower concentrations show larger chiral effects than those which act at higher concentrations. In addition, the twofold differences we have found for the two isomers acting on the sensitive channels are roughly what one might expect on the basis of Pfeiffer's rule. Because the relative potencies of the isomers vary from channel to channel, it is possible that their pharmacological profiles may also differ, with the anesthetic end point and associated side effects being differentially affected. Because safety factors (*14*) for inhalational anesthetics are very low (roughly 2 to 4), even the relatively small degree of stereoselectivity that we have observed could result in one of the enantiomers offering a significant clinical advantage over the racemic mixture. However, it should be stressed that general anes-

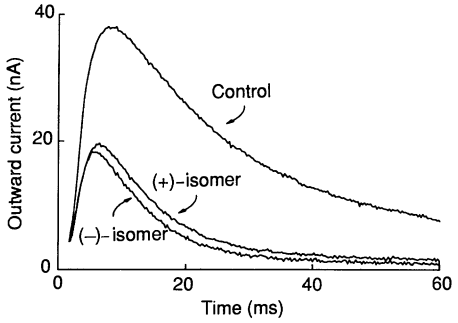

Fig. 3. The (−)-isomer of isoflurane is slightly more effective than the (+)-isomer at inhibiting the fast transient potassium current I_A. At the EC_{50} concentration for this current of 0.031 atm (2.4 MAC), the ratio of the inhibited currents remaining in the presence of (−)-isoflurane and (+)-isoflurane, respectively, was $I^-/I^+ = 0.95 \pm 0.01$ (mean ± SEM, $n = 20$). The I_A current was elicited by first holding the membrane potential at −100 mV and then depolarizing to −35 mV for 60 ms. The peak outward current was largely (~90%) blocked by 3 mM 4-aminopyridine. Measurements were made in normal saline with the same three identified neurons (*9*) used in the ACh experiments. The current record was filtered at 1 kHz. The capacitive transients at the start of the records have been excised for clarity.

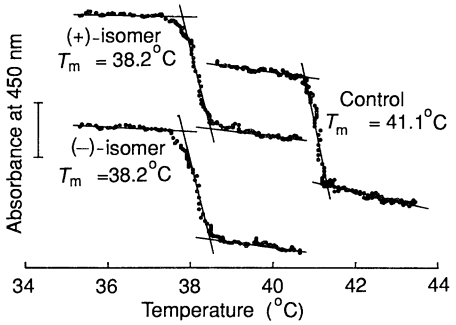

Fig. 4. The optical isomers of isoflurane have identical effects on lipid bilayers. The shift in the main chain–melting phase transition was the same for both isomers and the racemic commercial product Forane. An aqueous suspension of vesicles of dipalmitoyl lecithin (dipalmitoyl-L-α-phosphatidyl choline from Sigma) was made by adding 30 μl of a stock suspension to 3 ml of water with or without the isoflurane isomers (at 0.050 atm) in a 4-ml cuvette with a 1-cm pathlength. The stock suspension (30 mg/ml) was prepared by vigorously mixing dried lipid with water at a temperature (50°C) well above the chain-melting phase transition. The cuvette was placed in the heated stage of a Beckman DU8 spectrophotometer, and the absorbance at 450 nm measured as the temperature was increased at a rate of ~2°C per minute (*21*). The temperature was recorded with a thermocouple and a digital thermometer. The transition temperature T_m was defined as the midpoint in the abrupt step in absorbance. Multiple (four or more) determinations were made, and typical traces are shown. The lines were drawn by eye. The traces have been offset in the vertical direction for clarity. The calibration bar represents a change in absorbance of 0.04 units.

thetics most likely produce not only general anesthesia, but also their side effects, by binding to many different target sites. Therefore, effects of the enantiomers on the surgical patient (being due to many separate intermolecular interactions) are likely to be complex and are unlikely to mimic those found at any particular molecular target.

Although stereoselective behavior has previously been observed for intravenous agents such as ketamine (*15*) and barbiturates (*16*), it has rarely been looked for in volatile agents. A purification of halothane enantiomers was attempted many years ago (*17*), but only a partial separation was achieved; no stereospecificity was observed with the resulting mixtures (*18, 19*). The widespread belief that general anesthetics act through some "nonspecific" or "physicochemical" mechanism such as, for example, by expanding membranes by some critical volume (*20*), is difficult to reconcile with our observations of the stereospecificity of the isoflurane isomers which have, excepting their optical activity, identical physical properties. Although numerous physico-chemical mechanisms can be ruled out by our data, it is conceivable that lipid bilayers, which are composed of chiral components, could show some discrimination between the isoflurane optical isomers. For such small and apolar agents acting on a fluid lipid bilayer this seems unlikely, and, when looked for previously with the halothane enantiomers, it was not found (*18*). Nonetheless, we tested this possibility by measuring the depression of the melting point of lipid bilayers, a very sensitive test of bilayer disruption (*21*). Both isomers acted identically (Fig. 4) with the phase transition temperature being shifted from a control value of 41.2° ± 0.1°C (mean ± SEM, $n = 7$) to 38.2° ± 0.04°C (mean ± SEM, $n = 5$) by 0.050 atm of the (−)-isomer and to 38.2° ± 0.09°C (mean ± SEM, $n = 4$) by 0.050 atm of the (+)-isomer. Although lipid bilayers in nerve membranes, which are composed of heterogeneous components, might behave differently (*22*) and thus contribute to the observed biological stereospecificity, this seems most improbable in view of the different isomeric specificities, both qualitative and quantitative, found for the three different ion channels we have investigated. Coupled with previous observations (*1, 3*), our results support the hypothesis that general anesthetics cause their effects by binding directly to protein rather than by disrupting lipid bilayers.

REFERENCES AND NOTES

1. C. D. Richards, in *Topical Reviews in Anaesthesia*, J. Norman and J. G. Whitwam, Eds. (Wright, Bristol, UK, 1980), vol. 1, pp. 1–84; N. P. Franks and W. R. Lieb, *Nature* **300**, 487 (1982).
2. A. R. Dluzewski, M. J. Halsey, A. C. Simmonds, *Mol. Asp. Med.* **6**, 459 (1983); K. W. Miller, *Int. Rev. Neurobiol.* **27**, 1 (1985).
3. N. P. Franks and W. R. Lieb, *Trends Pharmacol. Sci.* **8**, 169 (1987).
4. J. Meinwald *et al.*, *Science* **251**, 560 (1991).
5. N. P. Franks and W. R. Lieb, *Nature* **333**, 662 (1988).
6. Electrophysiological measurements were made with the use of methods similar to those described previously (*5*). *Lymnaea stagnalis* snails (1 to 2 g) were obtained from Blades Biological (Cowden, Kent). Almost all of the measurements were made on cells completely isolated from the right parietal ganglion, which had been dissected away intact from the CNS. Identified neurons were isolated by impaling them with two microelectrodes and then slowly moving the ganglion away until the cell and its axons pulled free. Under these conditions, solution exchange around the cell was rapid and equilibration was generally complete in a few seconds or less. A two-electrode voltage clamp was used with the current record filtered by an eight-pole Bessel filter. The composition of normal saline was: NaCl, 50 mM; KCl, 2.5 mM; $CaCl_2$, 4 mM; $MgCl_2$, 4 mM; Hepes, 10 mM; and glucose, 5 mM. In TEA-cobalt saline, NaCl and $CaCl_2$ were replaced by 50 mM TEA-Cl (tetraethylammonium chloride) and 8 mM $CoCl_2$. The normal saline was titrated to pH 7.4 with NaOH and the TEA-cobalt saline titrated to pH 7.4 with TEA-OH. Solutions of the isoflurane isomers and the racemic mixture at chosen partial pressures were prepared with the appropriate fraction of a saturated solution, which was calculated by using the known saturated vapor pressure of isoflurane. These solutions were prepared immediately before the experiment, and care was taken throughout to minimize losses of the volatile anesthetics. Solutions containing cobalt were also prepared just before the experiments. All of the electrophysiological measurements were performed at 20° ± 1°C.
7. In order to confirm that the two optical isomers were present at identical concentrations, solubilities were determined in normal saline by gas chromatography. The solubilities were found to be 15.2 ± 0.3 mM for the (+)-isomer and 15.4 ± 0.2 mM for the (−)-isomer, which were not significantly different from the solubility (15.3 ± 0.2 mM) for the commercial racemic mixture (Forane).
8. A. Steward, P. R. Allott, A. L. Cowles, W. W. Mapleson, *Br. J. Anaesth.* **45**, 282 (1973).
9. These cells were in a group of three that had a yellow-speckled appearance and lay adjacent to one another and to the right of the neurosecretory region in the ventral portion of the right parietal ganglion.
10. This response had characteristics of an ACh-induced chloride current [J. Kehoe, *J. Physiol.* **225**, 115 (1972); N. K. Chemeris, V. N. Kazachenko, A. N. Kislov, A. L. Kurchikov, *ibid.* **323**, 1 (1982)] that has previously been shown to be sensitive to the volatile anesthetic enflurane [H. Arimura and Y. Ikemoto, *Br. J. Pharmacol.* **89**, 573 (1986)] and various intravenous agents [I. L. Cote and W. A. Wilson, *J. Pharmacol. Exp. Ther.* **214**, 161 (1980); S. E. Judge and J. Norman, *Br. J. Pharmacol.* **75**, 353 (1982)]. The current was activated by nicotine (10 μM) and the nicotinic agonist 1,1-dimethyl-4-phenolpiperazinium (10 μM) and was halfway inhibited by 1 μM tubocurarine (at 200 nM ACh). With K_2SO_4 electrodes, the reversal potential was about −60 mV, close to the expected chloride equilibrium potential. However, our experiments were performed with KCl electrodes, which shifted the reversal potential to about −10 mV and allowed large ACh-induced currents to be recorded in the region −70 to −80 mV, where contributions from $I_{K(An)}$ were minimized.
11. The simplest way to explain the dose-response data in Fig. 2 is to assume [N. P. Franks and W. R. Lieb, *Nature* **310**, 599 (1984)] that two molecules of anesthetic can bind independently to the ACh receptor-channel, each with the same dissociation constant, but that the presence of only one molecule is sufficient to cause inhibition. (The inhibition is assumed to be noncompetitive with respect to ACh.) If the dissociation constant is K_i, it is easy to show that the ratio I_c/I of control to inhibited currents is $(1 + C/K_i)^2$, where C is the concentration of the anesthetic. Thus, a value for K_i can be obtained

directly from a plot of the square root of I_o/I versus the concentration C.
12. If K_i^+ and K_i^- are the dissociation constants for the (+)-isomer and (−)-isomer, respectively, the mean dissociation constant for the racemic mixture is given by $2K_i^+K_i^-/(K_i^+ + K_i^-)$.
13. C C. Pfeiffer, *Science* **124**, 29 (1956).
14. B. Wolfson, W. D. Hetrick, C. L. Lake, E. S. Siker, *Anesthesiology* **48**, 187 (1978); R. B. Weiskopf *et al.*, *ibid.* **70**, 793 (1989).
15. M. P. Marietta, W. L. Way, N. Castagnoli, A. J. Trevor, *J. Pharmacol. Exp. Ther.* **202**, 157 (1977); P. F. White, J. Ham, W. L. Way, A. J. Trevor, *Anesthesiology* **52**, 231 (1980).
16. L.-Y. M. Huang and J. L. Barker, *Science* **207**, 195 (1980); M. K. Ticku, *Brain Res.* **211**, 127 (1981); P. Skolnick, K. C. Rice, J. L. Barker, S. M. Paul, *ibid.* **233**, 143 (1982); P. R. Andrews and L. C. Mark, *Anesthesiology* **57**, 314 (1982); G. Wahlström and L. Norberg, *Brain Res.* **310**, 261 (1984).
17. F. Y. Edamura, E. R. Larsen, H. M. Peters, paper presented at the 159th National Meeting of the American Chemical Society, abstract, Orgn 84 (1970).
18. J. J. Kendig, J. R. Trudell, E. N. Cohen, *Anesthesiology* **39**, 518 (1973).
19. L. H. Laasberg and J. Hedley-Whyte, *J. Biol. Chem.* **246**, 4886 (1971).
20. K. W. Miller, W. D. M. Paton, R. A. Smith, E. B. Smith, *Mol. Pharmacol.* **9**, 131 (1973).
21. M. W. Hill, *Biochim. Biophys. Acta* **356**, 117 (1974).
22. J. K. Alifimoff, L. L. Firestone, K. W. Miller, *Anesthesiology* **66**, 55 (1987).
23. We thank G. Vernice, D. Halpern, G. Huang, and L. Rozov of Anaquest Inc. for stimulating discussions and for supplying the isomers, S. Cull-Candy for helpful information, R. Dickinson for assistance with the phase transition measurements, and D. Blow for his comments on the manuscript. We are grateful to the MRC, the NIH (grant GM 41609), Anaquest Inc., and the BOC Group Inc. for support.

11 March 1991; accepted 9 July 1991

Enhancement of Growth of Virulent Strains of *Escherichia coli* by Interleukin-1

REUVEN PORAT, BURTON D. CLARK, SHELDON M. WOLFF, CHARLES A. DINARELLO*

Interleukin-1 (IL-1) is a polypeptide cytokine that mediates many physiological responses to infection and inflammation and is a growth factor for certain mammalian cells. Virulent and avirulent clinical isolates of *Escherichia coli* were grown in culture media in the presence of human IL-1. IL-1β, but not tumor necrosis factor or IL-4, enhanced the growth of virulent, but not avirulent, *E. coli*. This enhancement was blocked by the IL-1 receptor antagonist (IL-1ra). Radiolabeled IL-1 bound to virulent but not avirulent *E. coli* in a specific and saturable fashion; IL-1ra inhibited this binding. Thus, human IL-1 may recognize a functional IL-1–like receptor structure on virulent *E. coli* and may be a virulence factor for bacterial pathogenicity.

THE BIOLOGICAL ACTIVITIES OF IL-1 on mammalian cells are initiated by specific receptor binding (*1*). There are two human IL-1 receptors which are members of the immunoglobulin superfamily (*2*). IL-1 is a major mediator of septic shock (*3*); blockade of the IL-1 receptors prevents shock and death as a result of exposure to either lipopolysaccharide or *E. coli* bacteria (*4*). In this study we investigated how bacteria would respond to mammalian IL-1.

Various clinical isolates of *E. coli* were screened for sensitivity or resistance to the killing (bactericidal) activity of human serum from healthy individuals (*5*). Bacterial resistance to serum is a major virulence factor; >90% of the Gram-negative bacilli isolated from blood are serum-resistant (virulent), whereas most of the Gram-negative bacilli in the human gastrointestinal tract are killed by human serum, and therefore, are considered avirulent (*5, 6*).

Department of Medicine, Division of Geographic Medicine and Infectious Diseases, Tufts University, New England Medical Center Hospitals, Boston, MA 02111.

*To whom correspondence should be addressed.

Escherichia coli isolates (7) were tested for sensitivity or resistance to killing by normal human serum (*8*). Six virulent and four avirulent strains were characterized by their ability to survive when exposed to human serum; survival rates were >95% and <5%, respectively. When incubated in the presence of human IL-1β (*9*), we observed an increase in colony-forming units (CFU) of virulent but not avirulent strains. The growth rate of one of these virulent strains is shown in Fig. 1A. IL-1β at 10 and 100 ng/ml significantly enhanced growth compared to the control (heat-inactivated IL-1, $P < 0.001$ and $P < 0.0001$, respectively). The growth of an avirulent strain (Fig. 1B) was not affected by IL-1β, even at 100 ng/ml. Tumor necrosis factor (TNF) and IL-4 (1 to 1000 ng/ml) did not enhance growth of either avirulent or virulent bacteria (*10*).

We investigated the effect of IL-1 on bacterial growth in RPMI-1640, a defined tissue culture medium. The growth-promoting effect of IL-1β over control (no IL-1) was observed during the log phase of growth, before reaching the stationary phase ($P < 0.0001$); IL-1α had a smaller ($P < 0.05$) effect (Fig. 2A). No differences were detected in growth of the avirulent strain, with or without IL-1β (Fig. 2B).

The IL-1 receptor antagonist (IL-1ra) is a naturally occurring cytokine that binds to IL-1 receptors without manifesting agonist activities (*11*). IL-1ra blocked 75 to 85% of the IL-1–induced increase in growth rate. This blockade by the antagonist was observed at a 100-fold molar excess of IL-1ra to IL-1 (Fig. 2C). We next studied the ability of IL-1ra to block IL-1–induced bacterial growth enhancement at different molar ratios of IL-1ra to IL-1 (Fig. 3). At a molar ratio of IL-1ra to IL-1β of 1, there was a consistent but not statistically significant reduction; at ratios of 10:1 and 100:1, progressively greater inhibition of IL-1β–induced growth was observed ($P < 0.0005$ and $P < 0.00005$, respectively). Increasing the IL-1ra concentration to 1000-fold molar excess over IL-1β did not reverse the effect of IL-1 further (*10*), which retained ~20% of its growth-enhancing properties.

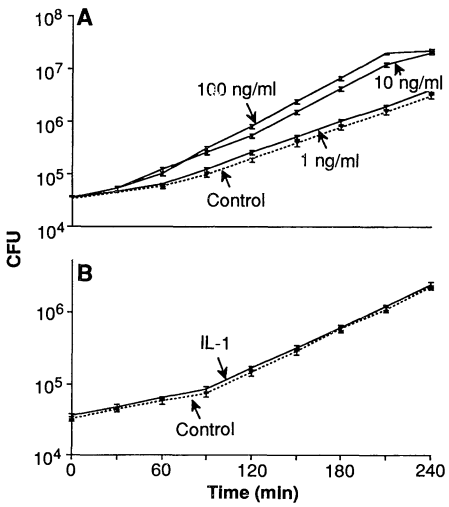

Fig. 1. Growth of *E. coli* in culture media in the presence of IL-1β. Bacteria were grown in brain heart infusion (BHI) broth for 16 to 18 hours at 37°C, washed, and resuspended in BHI to a final concentration of 10^6 bacteria per milliliter. Virulent and avirulent bacteria (3×10^4) were incubated in rotating Eppendorf tubes at 37°C in BHI in the presence of various concentrations of IL-1β or control (heat-inactivated IL-1, 90°C, 30 min). The cultures were diluted and plated on agar for bacterial counts at 30-min intervals from time 0 to 4 hours of incubation. The number of CFU was determined after 18 hours of incubation. For statistical analysis, the log of bacterial counts between 60 and 180 min (log phase growth before reaching stationary phase) was plotted against time, and a least squares fit was used to determine the slopes. The differences in growth rates of bacteria were then assessed by comparing these slopes (*16*). The results in panels A and B represent the mean ± SD of three experiments performed in duplicate. (**A**) Growth of virulent bacteria in the presence of various concentrations of IL-1β. (**B**) Growth curve of an avirulent strain in the presence of IL-1β (100 ng/ml).