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Interleukin 1 receptor antagonist blocks somnogenic and pyrogenic responses to an interleukin 1 fragment

(sleep/fever/rat/rabbit/behavior)

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ABSTRACT **Previously it was shown that human interleukin 1 (huIL-1) and a huIL-1 fragment, huIL-1β208–240, are somnogenic and pyrogenic in rabbits. However, the amino acid sequences for IL-1 are species-specific and it was unknown whether rabbit (rb) IL-1β208–240 and rat (rt) IL-1β208–240 were active in their respective species. Furthermore, it was unknown whether these fragments elicited their effects via the IL-1 receptors. Two doses of rbIL-1β208–240 (6.0 and 12.0 nmol) were intracerebroventricularly administered to rabbits. The 6.0-nmol dose had little effect, whereas the 12.0-nmol dose greatly increased non-rapid-eye-movement sleep across a 6-hr recording period and induced a febrile response. Rats injected intracerebroventricularly with rtIL-1β208–240 at dark onset responded to three doses of the peptide (1.2, 2.4, and 4.8 nmol). The 1.2-nmol dose did not greatly affect sleep but did induce a moderate febrile response. The 2.4- and 4.8-nmol doses increased non-rapid-eye-movement sleep across the 12-hr recording period. Maximal brain temperature elevations relative to controls after the 2.4- and 4.8-nmol doses of the peptide were 0.9 ± 0.2°C and 0.7 ± 0.2°C, respectively. These responses in both rabbits and rats were completely blocked or significantly attenuated when the animals were pretreated with an IL-1 receptor antagonist. These results suggest that the biological activities of IL-1β208–240 are mediated via the IL-1β receptors.**

Interleukin-1 (IL-1) promotes non-rapid-eye-movement sleep (NREMS) in rabbits, rats, and cats (reviewed in ref. 1). Much evidence now suggests that IL-1 is also involved in normal, physiological sleep regulation. For example, substances that induce IL-1 production enhance sleep, whereas substances that inhibit IL-1 production and/or actions inhibit sleep (reviewed in ref. 1). Plasma concentrations of IL-1 peak at the onset of NREMS in humans (2, ||), and in cats, concentrations of IL-1 in cerebrospinal fluid (CSF) vary in phase with sleep/wake cycles (3). Furthermore, anti-rabbit IL-1 antibodies reduce sleep in normal rabbits (M.R.O. and J.M.K., unpublished data). In addition to its effects on sleep, IL-1 possesses many biological activities. For example, it is a key immune-response modifier (reviewed in ref. 4), and it is likely that increased IL-1 production during infection is associated with sleep responses to microbial challenge (5, 6). Previously, specific fragments of IL-1 were reported to possess some, but not all, biological activities of the parent molecule. Thus, human (hu) IL-1β208–240 [208–240 refers to amino acid sequence of pro-IL-1 (4)] is somnogenic and pyrogenic in rabbits but does not stimulate thymocyte proliferation *in vitro* (7). Whether similar fragments corresponding to species-specific amino acid sequences also possessed biological activity, and whether those IL-1 fragment-induced activities were mediated by IL-1 receptor-dependent mechanisms, was unknown.

A specific IL-1 receptor antagonist (IL-1ra) has been cloned and characterized (8, 9). The IL-1ra is structurally related to IL-1, sharing about 25% amino acid sequence homology (8). IL-1ra blocks many actions of IL-1β (reviewed in ref. 10) and transiently reduces sleep in normal rabbits (11). It was of interest, therefore, to determine whether IL-1ra could block IL-1β208–240-induced responses *in vivo*. We now report that the somnogenic and pyrogenic responses of rabbits and rats to IL-1β208–240 are greatly attenuated or blocked by IL-1ra.

METHODS

Peptides. Rabbit (rb) IL-1β208–240 and rat (rt) IL-1β208–240 were synthesized by J.M.S. (Fig. 1). Aliquots of rbIL-1β208–240 were dissolved in dimethyl sulfoxide and stored at −20°C until use. Prior to injection into rabbits, the aliquots were brought to the appropriate volume with pyrogen-free saline so that the concentration of dimethyl sulfoxide injected into brain was ≤5%. rtIL-1β208–240 was dissolved in pyrogen-free saline. IL-1ra was a gift from Synergen (Boulder, CO). Frozen aliquots of IL-1ra were brought to the appropriate volume with pyrogen-free saline just prior to injection.

Rabbits. *Pasteurella*-free male New Zealand White rabbits (≈3.5 kg) received a lateral cerebral ventricular guide cannula, a thermistor, and stainless steel electroencephalographic (EEG) electrodes as previously described (11). In brief, the guide cannula for intracerebroventricular (ICV) injections was stereotactically placed in the left lateral ventricle. EEG electrodes were placed over the frontal and parietal cortices, and a calibrated 30-kΩ thermistor (model 44008, Omega Engineering, Stamford, CT) to measure brain temperature (Tbr) was implanted on the dura mater over the parietal cortex. The insulated leads from the thermistor and the EEG electrodes were routed to a Teflon pedestal (Plastics One, Roanoke, VA) that was attached to the skull with dental acrylic (Duz-All, Coralite Dental Products, Skokie, IL). Two weeks of postoperative recovery were allowed before the animals were used.

The rabbits were housed on a 12 hr/12 hr light/dark cycle (lights on at 0600) at 21°C. The animals were habituated for at least two 24-hr sessions to the recording chambers (Hotpack 352600, Philadelphia) prior to experimentation. The recording chambers were maintained on the same light/dark

Abbreviations: IL-1, interleukin 1; IL-1ra, IL-1 receptor antagonist; hu, human; rb, rabbit; rt, rat; EEG, electroencephalographic; ICV, intracerebroventricular(ly); Tbr, brain temperature; NREMS, non-rapid-eye-movement sleep.

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	208											219
Human	Lys	Lys	Lys	Met	Glu	Lys	Arg	Phe	Val	Phe	Asn	Lys
Rat	Lys	Lys	Lys	Met	Glu	Lys	Arg	Phe	Val	Phe	Asn	Lys
Rabbit	Lys	Lys	Lys	Met	Glu	Lys	Arg	Phe	Val	Phe	Trp	Lys
Consensus	*Lys*	*Lys*	*Lys*	*Met*	*Glu*	*Lys*	*Arg*	*Phe*	*Val*	*Phe*	---	*Lys*
	220									229		231
Human	Ile	Glu	Ile	Asn	Asn	Lys	Leu	Glu	Glu	Glu	Ser	Ala
Rat	Ile	Glu	Val	Lys	Thr	Lys	Val	Glu	Phe	Glu	Ser	Ala
Rabbit	Ile	Glu	Ile	Lys	Asp	Lys	Leu	Glu	Phe	Glu	Ser	Ala
Consensus	*Ile*	*Glu*	---	---	---	*Lys*	---	*Glu*	---	*Glu*	*Ser*	*Ala*
	232								240			
Human	Gln	Phe	Pro	Asn	Trp	Tyr	Ile	Ser	Thr			
Rat	Gln	Phe	Pro	Asn	Trp	Tyr	Ile	Ser	Thr			
Rabbit	Gln	Phe	Pro	Asn	Trp	Tyr	Ile	Ser	Thr			
Consensus	*Gln*	*Phe*	*Pro*	*Asn*	*Trp*	*Tyr*	*Ile*	*Ser*	*Thr*			

FIG. 1. Amino acid sequences of human, rat, and rabbit synthetic IL-1β208–240 peptide fragments.

cycle and the same temperature regime as the animal facility, and food and water were available ad libitum.

Apparatus and recording. The rabbit recording chambers contained an electronic swivel (Plastics One, Roanoke, VA) suspended by shock-absorbing coils. The swivel was connected via a flexible tether to the Teflon pedestal on the rabbit's head, allowing the animal relatively unrestricted movement within the cage. Body movements were detected by custom-built ultrasonic detectors (Biomedical Instrumentation, University of Tennessee). The leads from the electronic swivel and movement detectors were routed to Grass 7D polygraphs in an adjacent room. The EEG, Tbr, and body movement were recorded simultaneously from each animal. In addition, the EEG signal was band-pass-filtered and the 0.5–3.5 Hz (δ) and 4.0–7.5 Hz (θ) frequency bands were rectified and averaged. The ratio of EEG θ activity to δ activity was computed and displayed on the polygraph simultaneously with the EEG to facilitate scoring of vigilance states. Tbr values were also recorded at 10-min intervals by a data logger (Acrosystems 400, Beverly, MA). Determination of state of vigilance was made by visual scoring of the EEG in 12-sec epochs, using criteria previously reported (11).

Experimental protocol. The rabbits were placed in the recording chambers the evening prior to scheduled experiments. ICV injections were given the following morning, ≈3 hr after "lights on" (0900). The ICV injection volume was 25 μl; it was delivered over a 2-min period. Control recordings were made from each animal after injection of pyrogen-free saline containing dimethyl sulfoxide and compared with those made after injection of the peptide fragment. Thus, each animal served as its own control. Two doses of rbIL-1β208–240 were used, 6.0 and 12.0 nmol.

Rats. Adult male Sprague–Dawley rats (225–275 g) were anesthetized with pentobarbital sodium and implanted with EEG electrodes, a thermistor, and a guide cannula in a lateral cerebral ventricle as described above for rabbits, with modifications primarily for size. Habituation to recording procedures began 2 days after surgery. The animals were placed individually in recording cages, two cages in each environmentally controlled chamber (Hotpack 352600, Philadelphia). They were maintained on a 12 hr/12 hr light/dark cycle (lights on at 0500) at 25 ± 1°C with food and water available ad libitum. On the second postoperative day, the patency and free drainage of the guide cannula were verified by injecting 200–400 ng of angiotensin II (in 3–4 μl of pyrogen-free saline). Angiotensin II stimulates preoptic structures and elicits a drinking response (12), and this response served as an indicator of cannula patency. Only rats with a positive drinking response were included in the experimental protocol. The rats were then connected to the recording apparatus and were habituated by daily injection of pyrogen-free saline for 4 or 5 days before experiments began. On the sixth or seventh postoperative day, experimental recordings were initiated.

Apparatus and recording. The rats were attached to an electronic swivel in a manner similar to that of the rabbits, with the leads routed to a microcomputer-based analog/digital conversion system. The EEG, Tbr, and body movements were digitized, with the EEG sampled at 100.0 Hz, Tbr at 0.1 Hz, and body movement at 2.0 Hz. These data were stored directly on a computer disk until postacquisition analysis, at which time the EEG was visually scored in 30-sec epochs. Tbr was determined for each animal at 10-min intervals.

Experimental protocol. After the habituation period, the rats were injected with either pyrogen-free saline (control recording) or with one of three doses of the rtIL-1β208–240 peptide fragment (1.2, 2.4, or 4.8 nmol). The order of administration for the substances was varied and no rat was used for more than two doses of the peptide. Injection volumes of 4 μl were given over a 2-min period. The injections began 15 min prior to "lights off." Recordings started immediately after the lights went off and continued for the duration of the 12-hr dark period. At the end of the experimental protocol, the rats were again ICV injected with angiotensin II to verify cannula patency, and only data from those rats that responded by drinking were included in the subsequent analyses.

Double Injection Protocol (Rabbits and Rats). To determine whether responses to the synthetic peptides could be blocked by IL-1ra, separate groups of rabbits and rats were subjected to a double ICV injection protocol. Two ICV injections were spaced 10 min apart, and the volume for each injection was reduced to 15 μl for rabbits and to 3 μl for rats. Each animal was subjected to three treatments. First, control recordings were made after the double injection of appropriate vehicles. Second, the effects of the synthetic peptides in these groups of animals were evaluated by injecting first the vehicle followed 10-min later by the peptide (12.0 nmol for rabbits; 2.4 nmol for rats). Finally, the animals were pretreated by first injecting IL-1ra (100 μg in rabbits; 10 μg in rats) followed 10-min later with either rbIL-1β208–240 or rtIL-1β208–240. Responses to ICV injection of the peptides were compared with the double-injection vehicle controls and with those values recorded when the animals were pretreated with IL-1ra before they received the peptide fragments.

Statistical Analyses. All data were analyzed with the SPSS^x Information Analysis System. Deviations from values obtained during control recordings within doses across indicated time periods were detected by use of Friedman's nonparametric two-way analysis of variance (ANOVA) for k-related samples. If significant departures from control were indicated, the Wilcoxon matched-pairs signed-ranks test was used to determine specific time blocks contributing to these departures. Analyses between doses, and of the responses to the three recording periods of the double injection protocol, were done with a one-way ANOVA followed by the least-significant-differences multiple range test. An α level of $P \leq 0.05$ was taken as indicating significant departure from control values.

Table 1. Changes in sleep and brain temperature of rabbits and rats in response to IL-1β208–240

Animal	Peptide, nmol	*n*	NREMS,* %		REMS,* %		Tbr,† °C	
			Cont.	Expt.	Cont.	Expt.	Cont.	Expt.
Rabbit	6.0	8	40.9 ± 1.8	41.8 ± 1.7	5.2 ± 0.5	6.9 ± 0.6‡	38.1 ± 0.2	38.0 ± 0.2
	12.0	7	41.1 ± 1.6	57.0 ± 2.7‡	4.7 ± 0.6	2.6 ± 0.5‡	37.8 ± 0.2	38.9 ± 0.3§
Rat	1.2	6	28.7 ± 2.0	29.0 ± 2.1	6.1 ± 0.8	7.5 ± 0.8	37.9 ± 0.3	38.3 ± 0.3§
	2.4	7	28.5 ± 1.9	33.8 ± 2.3‡	7.4 ± 0.9	6.2 ± 0.7	37.3 ± 0.3	38.2 ± 0.4§
	4.8	7	28.4 ± 2.1	36.2 ± 2.2‡	5.2 ± 0.7	6.8 ± 0.8	37.4 ± 0.2	38.2 ± 0.3§

*Values are means ± SEM of percent time spent in each vigilance state across the 6- and 12-hr recording periods for rabbits and rats, respectively. Cont. = ICV injection of vehicle; Expt. = ICV injection of peptide fragments.

†Values are means ± SEM of maximum Tbr after ICV injection of the peptide fragments (Expt.) and of Tbr values recorded during corresponding control periods (Cont.).

‡$P < 0.05$ vs. Cont.; Friedman's non-parametric two-way analysis of variance.

§$P < 0.05$ vs. Cont.; Wilcoxon matched-pairs signed-ranks test.

RESULTS

IL-1β208–240. *Rabbits.* rbIL-1β208–240 induced an increase in the amount of time spent in NREMS (Table 1). The low dose of the peptide (6.0 nmol) had little effect on sleep, though it induced a slight increase in REMS (Table 1). The 12.0-nmol dose had profound effects on sleep. The amount of time spent in NREMS after this high dose increased by about 1 hr within the 6-hr recording period relative to control values. There was a relatively long latency before the enhanced NREMS was observed; though there was an increase in NREMS during the first 3-hr postinjection, it was not statistically significant. In postinjection hours 4–6 the increase in NREMS duration was much greater. Concurrent with the increases in duration of NREMS induced by the 12.0-nmol dose, there was a reduction in amount of time spent in REMS (Table 1). This effect on REMS followed a time course similar to that of increased NREMS; there was a tendency for reduced REMS in the first 3 hr postinjection that was not significant, followed in the second 3-hr time block by a significant reduction in REMS.

rbIL-1β208–240 also induced fever. Moderate fevers were evident in the first postinjection hour (maximum Tbr during this period was 0.3 ± 0.1°C greater than control values). After that, Tbr increased gradually throughout the recording period, reaching a maximal elevation of 1.1 ± 0.2°C relative to control values in hour 6.

Rats. ICV injection of rtIL-1β208–240 into rats induced increases in the amount of time spent in NREMS (Table 1). The lowest dose of the peptide injected into rats (1.2 nmol) did not greatly affect sleep across the 12-hr dark period. The 2.4-nmol dose of the peptide had a much greater effect on NREMS (Table 1); most of the increase in NREMS induced by this dose was recorded during postinjection hours 7–9. NREMS was also significantly increased after ICV injection of the 4.8-nmol dose of the peptide (Table 1). The time course of increased NREMS after this dose was essentially the same as that after the 2.4-nmol dose; maximal enhancement of NREMS occurred in postinjection hours 7–9. rtIL-1β208–240 did not affect REMS in rats (Table 1).

rtIL-1β208–240 induced moderate febrile responses after ICV injection of each of the doses tested. Maximal Tbr elevation relative to the control value after ICV injection of the 1.2 nmol dose was about 0.4°C (Table 1); it occurred in postinjection hour 5. Maximal Tbr elevations relative to control values after the 2.4- and 4.8-nmol doses of the peptide were 0.9 ± 0.2°C and 0.7 ± 0.2°C, respectively. The time courses of these febrile responses paralleled those of NREMS responses; maximal Tbr elevations were in postinjection hours 8–9.

IL-1ra Pretreatment. *Rabbits.* The group of rabbits that was subjected to the double ICV injection protocol responded to 12.0-nmol of rbIL-1β208–240 in a manner similar to that of the group subjected to a single ICV injection only. The amount of time spent in NREMS increased significantly across the 6-hr recording period, and REMS was significantly reduced. The increases in NREMS were not apparent until the third postinjection hour, and then they persisted throughout the remainder of the recording period (Fig. 2). The time spent in REMS was significantly reduced during postinjection hours 4–6 (Fig. 2). rbIL-1β208–240 also induced fevers in this group of rabbits, with maximal Tbr elevation of 0.8 ± 0.2°C relative to control values occurring in hour 5 (Fig. 2).

When the rabbits were pretreated with 100 μg of IL-1ra, the NREMS responses to 12.0 nmol of rbIL-1β208–240 were absent (Fig. 2). In contrast, pretreatment with IL-1ra failed to affect the inhibition of REMS induced by rbIL-1β208–240 (Fig. 2).

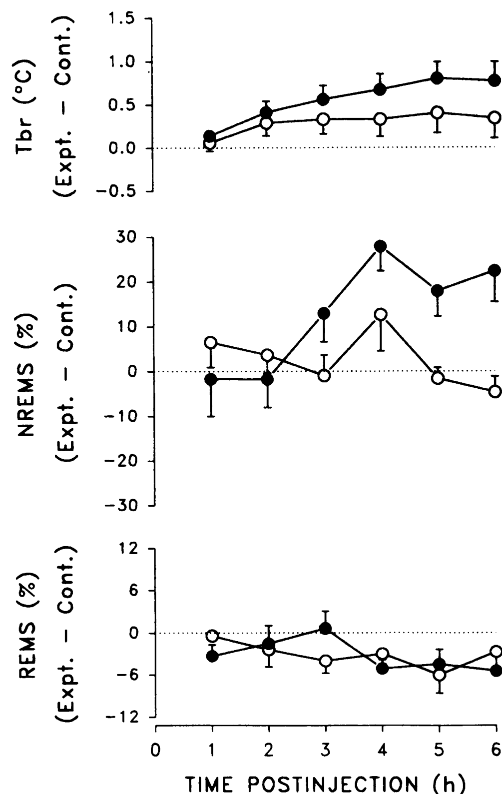

FIG. 2. Somnogenic and pyrogenic responses of rabbits to rbIL-1β208–240 and the effective inhibition of those responses by pretreatment with IL-1ra. Values are means ± SEM for 7 rabbits and are expressed as change relative to values recorded after double vehicle control injections (Expt. = values recorded after injection of test substances; Cont. = values recorded after double vehicle control injections represented by the dotted 0 line). Recordings were made for 6 hr during the light period of the light/dark cycle. ●, Vehicle plus 12.0 nmol of rbIL-1β208–240; ○, 100 μg of IL-1ra plus 12.0 nmol of rbIL-1β208–240.

Pretreatment of rabbits with IL-1ra also attenuated the fevers induced by the IL-1β208–240 peptide (Fig. 2).

Rats. In general, rats responded to 2.4 nmol of the synthetic peptide in the double injection protocol in a manner similar to those animals that were subjected to this dose of the peptide in the single injection protocol. NREMS was increased after a relatively long latency, REMS was not significantly affected, and a moderate febrile response developed (Fig. 3).

Pretreatment with 10 μg of IL-1ra completely blocked the NREMS and Tbr responses to 2.4 nmol of rtIL-1β208–240 peptide (Fig. 3). Not only was the expected increase in NREMS elicited by the peptide eliminated, but the rats spent significantly less time in NREMS across the 12-hr recording period after 10 μg of IL-1ra followed by 10 nmol rt IL-1β208–240 than they did after the double vehicle injection control recordings. REMS was not affected by pretreatment with IL-1ra.

DISCUSSION

Previously it was reported that native IL-1β, recombinant huIL-1β, and huIL-1β208–240 were somnogenic and pyrogenic in rabbits (reviewed in ref. 1). These observations are now extended to include responses of rabbits and rats to IL-1β208–240 synthesized from species-specific amino acid sequences. The sleep and febrile responses induced by these peptide fragments appear to be mediated by the IL-1 receptors.

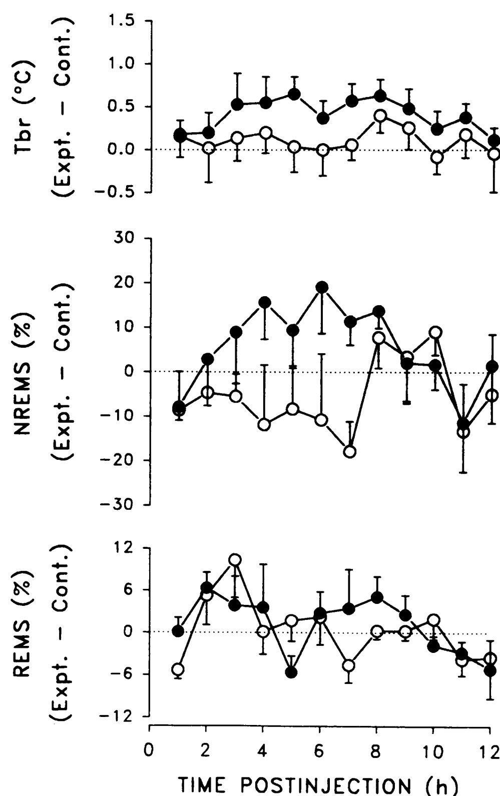

FIG. 3. Changes induced in brain temperature and sleep–wake activity of rats by 2.4 nmol of rtIL-1β208–240, and the blockade of those actions by pretreatment with IL-1ra. Values are means ± SEM for 6 rats and are expressed as change relative to values recorded after double vehicle control injections (Expt. = values recorded after injections of test substances; Cont. = values recorded after double vehicle control injections represented by the dotted 0 line). Injections were timed just prior to "dark" onset and the 12-hr recordings continued for the duration of the dark period of the light/dark cycle. •, Vehicle plus 2.4 nmol of rtIL-1β208–240; ○, 10 μg of IL-1ra plus 2.4 nmol of rtIL-1β208–240.

The rt- and rbIL-1β208–240 fragments differ, in part, from mature huIL-1β in their effects in rats and rabbits. On a molar basis, the dose of the IL-1β208–240 fragments necessary to elicit a somnogenic or pyrogenic response is much greater than that of recombinant huIL-1β. That peptide fragments are less active than the parent molecules appears to apply to other fragments as well (7, 13, 14); the reasons for this finding are not completely understood, but it is possible that larger ligands form more stable ligand–receptor complexes. It is also possible that smaller molecules are more easily hydrolyzed and/or diffuse away from the target cell surface more rapidly.

Another difference between the IL-1β208–240 fragments and mature recombinant huIL-1β is the delay between the time of injection and the first signs of somnogenic and pyrogenic responses of the animal. Latency to febrile and somnogenic responses elicited by the peptide fragments was 2–3 hr, whereas responses to recombinant huIL-1β become manifest in the first postinjection hour in both rabbits and rats (15, 16). The reasons for these differences are unknown. It may be that the rt- and rbIL-1β208–240 peptide fragments are not intrinsically somnogenic or pyrogenic but may have to induce production of IL-1 and/or other somnogenic cytokines. IL-1 induces its own production (17) as well as the production of other somnogenic cytokines [e.g., tumor necrosis factor (4)].

Some of the biological activities of IL-1 may be localized to specific regions of the mature molecule. For example, huIL-1β208–240 is somnogenic and pyrogenic in rabbits and stimulates prostaglandin E_2 production, yet does not induce T-cell proliferation (7). The huIL-1β163–171 peptide fragment is active in murine thymocyte proliferation assays and stimulates IL-2 production in spleen cells, yet does not induce prostaglandin E_2 synthesis *in vitro* and is not pyrogenic for mice *in vivo* (14). It may be then, that receptor–ligand endocytosis of these fragments is followed by cell-specific hydrolysis accounting for differential biological activities. Synthetic peptide fragments of other cytokines also exhibit subsets of the biological activities attributed to mature molecules. For example, a fragment of tumor necrosis factor α composed of amino acids 31–68 is pyrogenic and somnogenic in rabbits, whereas amino acids 69–100 induce a febrile response and suppress food intake without affecting sleep (20).

The concept that there is a cascade of events leading to specific responses induced by IL-1 is also indirectly supported by the fact that the biological actions of mature IL-1 may be dissociated from one another. For example, enhanced NREMS in response to IL-1 can be separated from the febrile response. Cyclooxygenase inhibitors and protein synthesis inhibitors, when administered systemically, block febrile responses to cytokines but do not block somnogenic responses. Therefore, it is not likely that the somnogenic responses to IL-1 are a direct consequence of fever. Changes in NREMS and REMS duration in response to IL-1 can also be separated. In rabbits, REMS is suppressed by doses of IL-1 that enhance NREMS, whereas in rats, REMS is unaffected by doses of IL-1 that enhance NREMS. In addition, in rabbits IL-1ra blocks increases in NREMS duration induced by IL-1 but does not reverse IL-1-induced suppression of REMS.

IL-1ra is capable of blocking many IL-1 actions both *in vivo* and *in vitro* (reviewed in ref. 10). IL-1ra binds to both IL-1 receptors, and has about the same affinity as IL-1β for IL-1 receptor type I (10). A consistent finding in studies on IL-1ra is that a molar excess is necessary to block IL-1 actions, both *in vivo* and *in vitro*. Few IL-1 receptors need to be occupied to trigger a biological response to IL-1 (10), and this may

explain the molar excess requirement for IL-1ra to block IL-1 actions. Our findings here are a notable exception. Thus, the IL-1β208–240 peptide fragment was in molar excess of IL-1ra, yet IL-1ra blocked the IL-1β208–240 effects. These data support the ideas presented above, that IL-1β208–240 may not form as stable a ligand–receptor complex as mature IL-1 or that the small peptide fragments diffuse and/or are hydrolyzed more rapidly than the mature molecule. Regardless of the reasons, our results strongly suggest that IL-1β208–240 effects are receptor-mediated. This conclusion is also supported by our previous *in vitro* findings that IL-1ra blocks IL-1β208–240-induced intercellular adhesion molecule 1 (ICAM-1) expression on human glioblastoma cells (L. Hong, M.R.O., and J.M.K., unpublished data).

The role of IL-1 in pathophysiology is well documented (4). We have hypothesized that IL-1 is also involved in normal, physiological sleep regulation, and the physiological and anatomical evidence to support an hypothesis of IL-1 involvement in sleep regulation has been extensively reviewed (1). If IL-1 is indeed involved in normal physiological sleep regulation, then IL-1ra might reduce sleep. IL-1ra does, in fact, transiently reduce NREMS in rabbits (11). However, the half-life of IL-1ra in blood is relatively short (<10 min), and endogenous IL-1 might quickly overcome the effects of exogenously administered IL-1ra. In the current study, pretreatment of rats with IL-1ra followed by the IL-1β208–240 peptide fragment resulted in reduced NREMS to values significantly below those obtained after vehicle control injections. This finding in itself provides support for the role of IL-1 in normal sleep. However, preliminary studies indicate that huIL-1ra does not affect sleep of normal rats (M.R.O. and J.M.K., unpublished data). In addition, IL-1 is but one part of an orchestration of molecular events proposed to be involved in sleep regulation (18). Responses to exogenous administration of cytokines and other substances, including IL-1ra, are dependent on the state of the system at the time of injections. For example, rats respond differentially to IL-1 depending on the time of day of injections; the same dose may result in either enhanced NREMS or reduced NREMS (16). This dichotomy is likely due to circadian fluctuation of concentrations of endogenous corticotropin-releasing hormone, a substance known to be of critical importance for moderation of IL-1 effects (19). Thus, when concentrations of corticotropin-releasing hormone are low, IL-1 administration stimulates negative feedback mechanisms resulting in increased wakefulness, whereas when concentrations of corticotropin-releasing hormone are already high, IL-1 administration may stimulate primarily sleep mechanisms.

The regulatory mechanisms for IL-1 actions and the interactions between IL-1 and other cytokines/neuroendocrines are complex and poorly understood. It is clear from current data, however, that peptide fragments of IL-1β are capable of eliciting somnogenic and febrile responses in rabbits and rats and that these actions appear to be receptor-mediated. Whether or not these biological activities are inherent in the fragments or are the result of stimulation of other mechanisms (e.g., synthesis of IL-1) remains to be determined.

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