STATEMENT

Synopsis of Research Report 128

HEALTH EFFECTS INSTITUTE

Neurogenic Responses of Rat Lung to Diesel Exhaust

Air pollution, including gases and particulate matter emitted from motor vehicles, has been associated with increases in both morbidity and mortality, but the underlying mechanisms responsible for these effects are not well understood. Insight into such mechanisms will aid in the understanding of human risk associated with air pollution.

In 1998, HEI issued Request for Preliminary Applications 98-6, entitled "Health Effects of Air Pollution". In response, Dr Witten and colleagues proposed to investigate the inflammatory effects of diesel exhaust exposure on rat airways. The investigators focused on the role of neurogenic inflammation, an inflammatory response defined by the release of neuropeptides, such as substance P (SP), from sensory nerve fibers known as *C fibers* located within the lung tissue. Neurogenic inflammation has been implicated in responses to inhaled irritants such as ozone and cigarette smoke and has been implied to play a role in asthma. HEI funded Dr Witten's study because they thought it would provide valuable information on the pathogenic mechanisms involved in respiratory responses to diesel exhaust.

APPROACH

The investigators exposed female rats (8 weeks old) to two concentrations of whole diesel exhaust emissions (35 and 630 $\mu g/m^3$ particulate matter) from a heavy-duty 1990s Cummins research engine. Exposures were conducted over 3 weeks (4 hr/day, 5 days/week); neurogenic and other inflammatory markers were measured immediately after the end of exposure. Half of the rats in each exposure group were pretreated with capsaicin, a neurotoxin that depletes sensory C fibers of neuropeptides and thereby inhibits the neurogenic inflammatory pathway. Control groups were exposed to air. Cigarette smoke exposure (~400 $\mu g/m^3$, 4 hr/day for 7 days) provided a positive control. The investigators measured endpoints of neurogenic inflammation: SP protein and gene expression, density of the SP receptor neurokinin-1 (NK1), and activity of neutral endopeptidase (NEP), the enzyme that breaks down SP. They also assessed leakage of blood plasma into lung tissue and other inflammatory markers, such as levels of the cytokines interleukin (IL)-1β, IL-6, IL-10, IL-12, and tumor necrosis factor α, numbers of inflammatory cells in lung tissue, and cellular lung pathology.

Witten collaborated with researchers from the University of Wisconsin to develop the diesel exhaust exposure system. Before animal exposures started, they evaluated a number of engine operation modes using the California Air Resources Board 8-mode test system for gaseous, particulate, and metal emissions. The investigators selected California Air Resources Board mode 6 for the animal experiments.

RESULTS AND INTERPRETATION

The authors are among the first to investigate neurogenic inflammation in the lungs of rats exposed to whole diesel exhaust. After exposure to both concentrations of diesel exhaust, consistently higher levels of plasma leakage and lower activity of the enzyme NEP were observed. Changes in levels of SP and its receptor NK1 were less consistent, however, and few changes were observed in cytokine levels. These results confirm previous findings of mild inflammatory responses after exposure to diesel exhaust.

The role of neurogenic inflammation remains unclear, however. Neurogenic inflammation has been convincingly demonstrated after exposure to ozone, sulfur dioxide, hydrogen sulfide, cigarette smoke, and wood smoke. In those studies, inflammatory responses were eliminated after animals had been treated neonatally with capsaicin. In the Witten study, rats were treated with capsaicin as

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This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr Mark L Witten at the University of Arizona, Tucson AZ. The complete report, *Neurogenic Responses in Rat Lungs After Nose-Only Exposure to Diesel Exhaust*, can be obtained from HEI or our website (see reverse side).

WITTEN 128

young adults. Witten and colleagues showed that capsaicin treatment caused a complete absence of SP in lung tissue, but it had little effect on the inflammatory response to diesel exhaust. Thus, the results do not support a role for C fibers in the airway inflammatory response to diesel exhaust. The results of the current study may also have been complicated by neuropeptide release from sources other than C fibers (such as the airway ganglia, mast cells, and eosinophils). The investigators did find evidence for neurogenic inflammation after exposure to cigarette smoke, which was in part reversed by capsaicin treatment.

When testing emissions at different engine operating conditions, the investigators found that elemental carbon dominated at medium to heavy loads, while organic carbon dominated emissions at lighter loads. Levels of sulfate, calcium, iron, magnesium, and particle numbers differed among operating conditions. They found some differences in particle composition between the lower and the higher level of diesel exhaust during the animal exposures, but their contribution to the inflammatory effects, if any, remains unclear. Additional research will be needed to investigate further the inflammatory mechanisms and the role of C fibers in airway responses to diesel exhaust and to identify which components of the diesel exhaust mixture may contribute to the inflammatory effects.

Neurogenic Responses in Rat Lungs After Nose-Only Exposure to Diesel Exhaust

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