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Exposure of rabbits to styrene

Electronystagmographic findings correlated to the styrene level in blood and cerebrospinal fluid

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LARSBY, B., THAM, R., ÖDKVIST, L. M., HYDÉN, D., BUNNFORS, I. and ASCHAN, G. Exposure of rabbits to styrene: Electronystagmographic findings correlated to the styrene level in blood and cerebrospinal fluid. *Scand. j. work environ. & health* 4 (1978) 60—65. Objective methods for critically evaluating the toxic effect of industrial solvents are highly desirable. As many of these solvents are suspected to cause vertigo, an animal experimental model was set up for studying the effects of solvents on the vestibular systems. The vestibular function was studied by registration of involuntary eye movements — nystagmus — which are elicited via central vestibulo-oculomotor connections. During exposure to styrene a so-called positional nystagmus was demonstrated that indicated vestibular disturbances. Nystagmus is normally elicited by rotatory acceleration. During exposure to styrene the direction of this rotatory nystagmus was reversed. The incidence of the positional nystagmus correlated well with the blood level of the solvent, measured by gas chromatography. Kinetic studies also demonstrated a rapid equilibration between the level of the solvent in arterial blood and cerebrospinal fluid, and therefore suggested that estimation of the arterial level reliably indicates the level in the central nervous system.

Key words: blood, cerebrospinal fluid, electronystagmography, gas chromatography, pharmacokinetics, rabbit, styrene, vestibular disturbances.

Styrene is a commonly used solvent in the plastic industry. Chemically it is closely related to xylene and toluene. Styrene is sparingly soluble in water but has a high lipid solubility. It has a characteristic irritating vapor. The most important route of absorption is via the lungs, but because of its high lipid solubility the percutaneous route can also be of importance. Besides pulmonary excretion styrene is also metabolized to mandelic and phenylglyoxylic acids (16).

Workers overexposed to styrene in plastic industries have shown test reaction times that are significantly longer than normal (12). Volunteers, not previously exposed to styrene, showed several signs of neurological impairment, like pathological results on the Romberg test, during exposure to 376 ppm of the solvent (21). After 1 h of exposure the volunteers noted subjective discomfort in the form of headache, nasal and eye irritation, and a feeling of slight inebriation.

Hence, it is rather evident that styrene has an undesirable effect on the central nervous system. Therefore it seemed urgent to include the substance in a pre-

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viously started study designed to develop reliable methods for experimentally investigating the effects of organic solvents on the central nervous system (CNS), specifically the vestibular (= balance) system.

This system is intimately connected to the oculomotor apparatus and can be studied by recordings of involuntary eye movements — a phenomenon called nystagmus. Nystagmus may be induced by several kinds of stimuli. Stimulation of the vestibular sense organ in the labyrinth by rotation of the subject elicits nystagmus via connections of the vestibular and oculomotor pathways within the CNS. Nystagmus may also be provoked by direct pharmacological stimulation of the central pathways.

We have previously demonstrated that both xylene and methylchloroform provoke a special kind of nystagmus — positional nystagmus (8, 15). This nystagmus is evident only when the animal is lying on its side. The nystagmus induced by xylene or methylchloroform correlates well to the arterial blood levels of the solvents. The aim of the present investigation was to test the sensitivity of the vesibular apparatus of rabbits to styrene with the use of the previously developed experimental model.

METHODS

Sixteen rabbits with a mean body weight of 3.1 kg (SD = 0.25 kg) were used. Electronystagmography was performed in an animal box which could be darkened by closure of the lid. In the box the rabbit's head was held steady by a headholder. The box was mounted on a Stille rotating platform for rotatory testing. The box could also be tilted to the right and left lateral positions. Recording electrodes consisting of subcutaneous needles were inserted laterally to the eyes, and one ground electrode was placed centrally on the forehead (4, 9). The signals were fed via an amplifier to an Elema Schönander Mingograph 34. Electronystagmographic recordings were performed every 10 min during the experiment with the rabbit in the prone position and in the right and left lateral positions. Rotatory acceleration tests were performed every 30 min. The acceleration was $5°/s^2$ to a top speed of $73°/s$. After a constant speed period of 60 s, a $5°/s^2$ deceleration to standstill followed.

An ear vein was cannulated and a polyethylene catheter was introduced far enough to reach the jugular vein. A 10 % solution of styrene in a lipid emulsion (Intralipid®) was administered through this catheter by continuous infusion. The rate of infusion was 3.1 mg to 12.6 mg of styrene per minute.

To collect blood samples, a polyethylene catheter was introduced into an ear artery. In four experiments blood was also taken from the inferior vena cava, reached via a smaller vein on the medial side of the upper part of the hind limb.

In the four experiments in which the concentration of the solvent in the cerebrospinal fluid (CSF) was analyzed, general anesthesia was intravenously induced by Nembutal (30—40 mg per kilogram of body weight), and maintained by Nembutal (2—4 mg/kg and hour) for 1 h either as a single anesthetic drug or combined with inhalation of nitrous oxide (70 % N_2O, 30 % O_2). The animals were artifically ventilated via a tracheostoma. End tidal carbon dioxide was kept between 3 and 4 %. Through a small incision in the skin the CSF space was cannulated suboccipitally with a needle, and a fine silastic tube was introduced. In these CSF experiments no electronystagmographic recordings were made.

For the styrene analyses 0.5 ml of blood or 0.1 ml of CSF was added to a glass stoppered tube containing a solution of 10 % v/v of isopropanol and 0.006 % v/v (blood analysis) or 0.0002 % v/v (CSF analysis) of xylene in water. The added amount of blood or liquid was weighed. Within 2 h, 0.1 ml of the mixture was transferred to a 1-ml glass vial which was sealed hermetically with a silicone-coated rubber membrane. The vial was shaken for 10 s and was then stored at room temperature for at least 30 min but not for more than 2 h. With an airtight syringe and a fine needle 0.1—0.2 ml of the gas phase was aspirated and injected into a Varian 1400 gas chromatograph, equipped with a 5 % OV-17 glass column (1.5 m x 2 mm inner diameter) and a flame ionization detector. The

column temperature was 74°C, and the detector temperature 200°C. For each experiment a calibration curve was constructed with the use of either blood taken from the rabbit before the experiment started or human CSF. Half a milliliter of the blood or 0.1 ml of CSF was added to test tubes containing the isopropanol-xylene-water solution and different amounts of styrene. The calculations were based on the peak height ratio styrene/internal standard (xylene). The reproducibility was tested by 30 analyses from the same sample of blood to which a known amount of styrene had been added. This test was performed at three different concentrations within the range occurring in the rabbit experiments.

RESULTS

Gas chromatographic analysis with the head space method revealed symmetrical peaks for the styrene and the internal standard (xylene). The retention times were about 1.1 and 1.7 min, respectively. The calibration curve was linear in the range of 1—100 ppm (milligrams of styrene per kilogram of blood or CSF). The SD_{rel} was 2 %.

After the start of the intravenous infusion there was a rapid rise of the styrene level in arterial blood during the first half hour, followed by a slower increase (fig. 1).

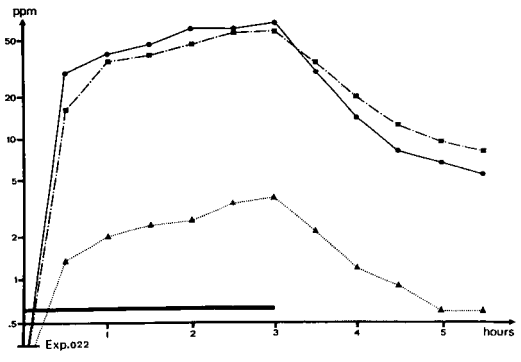

Fig. 1. Arterial (●——●) and central venous (■ · — · ■) blood concentrations and cerebrospinal fluid (▲ · · · ▲) concentrations in an experiment during and after intravenous infusion of 5 mg per minute of styrene. The bar indicates time of infusion.

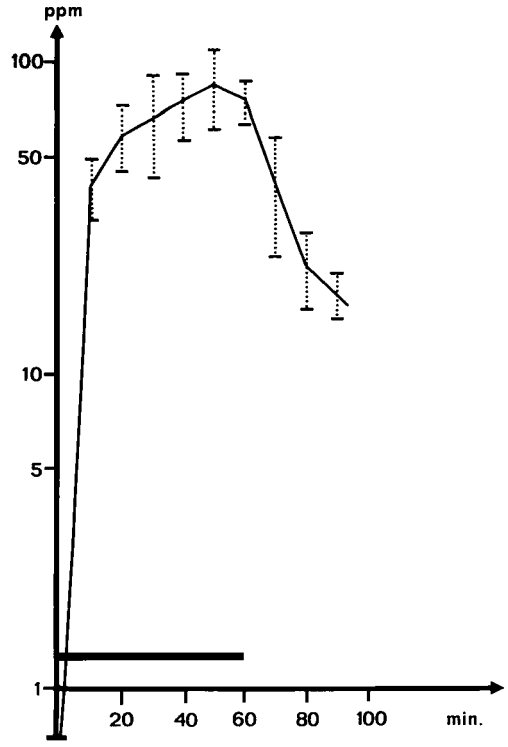

Fig. 2. Mean arterial blood concentration (± 1 SD), expressed in milligrams per kilogram of blood (ppm) during intravenous infusion of styrene (dissolved in Intralipid). The curve represents seven experiments with an infusion rate of 9.3 mg of styrene per minute. The bar indicates time of infusion.

The necessary time for reaching a nearly constant arterial level was more than 2 h with an infusion rate of 4.9 mg of styrene per minute. The arteriovenous difference for the solvent concentration was already small 30 min after the infusion was started, but in all of the experiments there still existed a difference of 5 to 10 % even after 3 h of infusion (fig. 1). After cessation of the infusion the clearance of the solvent was faster from arterial blood than from central venous blood.

The CSF level curve had the same shape as that of the blood level (fig. 1). The CSF concentration was constantly 5—10 % of the corresponding arterial concentration during the entire experiment.

The individual variations of the arterial blood levels during the infusion of 9.3 mg of styrene per minute is demonstrated in fig. 2.

None of the rabbits showed any positional nystagmus before the infusion was started. The incidence of positional nystagmus and the related arterial blood level of styrene 20 min after the start of infusion is presented in fig. 3. At arterial blood levels above 40 ppm almost all the rabbits (10 out of 11) showed a positional nystagmus, i.e., left beating in the right lateral position and right beating in the left lateral position (fig. 4). In the prone position none of the rabbits had any nystagmus.

In the preintoxication tests all of the rabbits showed a normal response to the rotatory acceleration, i.e., right beating nystagmus when accelerated clockwise and vice versa. They also responded to deceleration according to rule. During the styrene intoxication some of the rabbits (6 out of 16) showed a paradoxical rotatory response i.e., left beating nystagmus when rotated clockwise and vice versa (fig. 5).

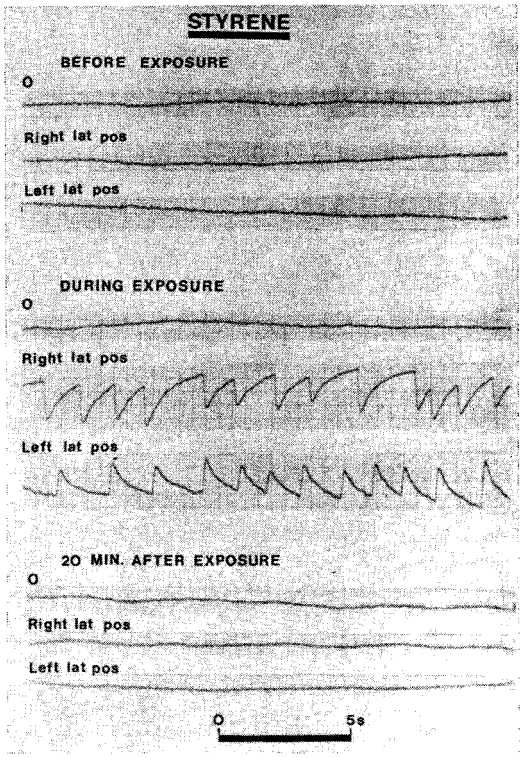

Fig. 4. Electronystagmographic recordings from a rabbit before, during, and after styrene exposure. Recordings were performed in upright, right lateral and left lateral positions. Upwards in the graph indicates an eye movement to the right.

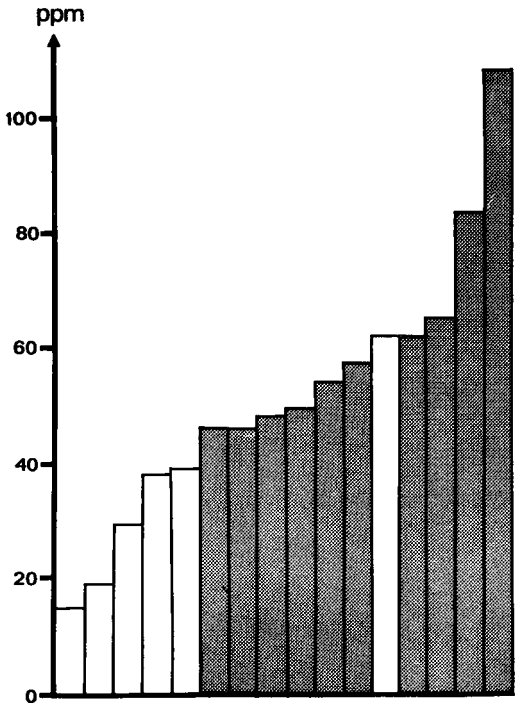

Fig. 3. Arterial blood concentrations of styrene and the incidence of positional nystagmus (dark columns) in 16 rabbits, 20 min after the start of styrene intravenous infusion. Each column represents one rabbit.

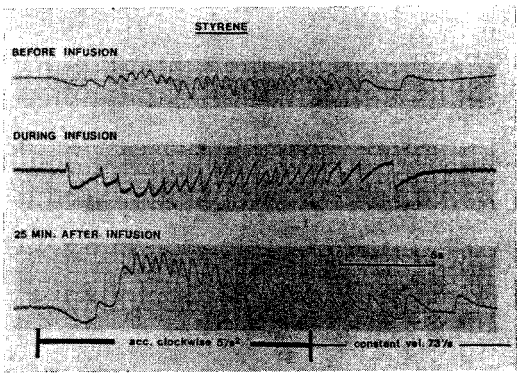

Fig. 5. Nystagmus responses to rotatory acceleration before, during and after styrene exposure. The acceleration period and the subsequent constant velocity rotation are indicated below.

DISCUSSION

Physiological rotatory nystagmus is induced in the three semicircular canals of the labyrinths. Each canal is filled by the inner ear fluid — endolymph — and each has a widening in which a cupula acts as a swing door sensing the pressure of the fluid when the head starts to rotate. An accelerated rotation clockwise should give rise to a right beating nystagmus. Ethyl alcohol diminishes the rotatory responses at low intoxications and abolishes the response at higher blood alcohol concentrations (14). Xylene on the other hand has an exaggerating effect on the phenomenon that thus indicates a different influence on the vestibular system than that of ethyl alcohol (8). The aromatic solvent may act on some part of the vestibulo-oculomotor servo (feed back) system, perhaps the efferent vestibular system. The effect of styrene, reversing the direction of rotatory nystagmus, is astonishing. A similar effect exhibited by any other drug has not, as far as we know, been described. To explain this finding further research is necessary.

Like alcohol (3, 4, 5, 9) and industrial solvents, such as xylene (8) and methylchloroform (15), styrene was shown to elicit a dose correlated positional nystagmus in rabbits. The direction of the alcohol induced nystagmus is in both man and rabbits initially left beating in the left lateral position and right beating in the right lateral position (3, 4, 5, 6, 7, 9). A theory explaining the alcohol nystagmus as being induced by a stimulation of the peripheral sense organ has been proposed (17, 18). However, nystagmus induced by styrene as well as by xylene and methylchloroform is left beating in the right lateral position and vice versa (8, 15). These findings and the mostly diverging effects of the different solvents on the rotatory responses cannot possibly be explained by a simple action in the inner-ear. A direct action on the central vestibulo-oculomotor pathways seems the only logical explanation.

Intravenous infusion of styrene dissolved in a lipid emulsion seems to be a reliable method for obtaining predictable and steady blood levels of the solvent in non-anesthetized rabbits. However, with a constant rate of styrene infusion, several hours were needed to reach relatively steady levels in arterial and venous blood. During the first hours the elimination of the solvent by the lungs and by metabolism was therefore less than the continuous supply by infusion. This finding is probably dependant on the high solubility of styrene in blood, the partition coefficient for blood/gas being 32 at 37°C (22). We have previously demonstrated that a relatively steady state was reached much faster for arterial blood level during the infusion of methylchloroform (15), the solubility of which is only 1.4 at 40° in blood (19). Our observations concerning the blood levels of the two solvents after intravenous infusion are in agreement with the investigations of Åstrand et al. on uptake during inhalation of these solvents in human experiments (1).

Although it takes a long time to achieve a balance between the intravenous supply and the elimination by metabolism and pulmonary expiration, the central venous concentration very rapidly approaches the arterial level and, therefore, probably indicates a rapid equilibration between the blood and the tissues of organs with a high blood supply like the brain (13). This supposition is further supported by the fact that the relationship between the concentration in blood and in CSF was constant during our experiment. The quotient was about 0.05—0.1, which probably represents the partition coefficient at equilibrium. A close correlation between the arterial blood level and the tissue concentration in the brain is also indicated in our experiments by the agreement of this level and the incidence of positional nystagmus.

According to Swedish law the highest permitted air content of styrene in industrial work is 50 ppm. In human experiments Åstrand et al. (2) demonstrated an arterial blood level of about 2 ppm after moderate work (50 W) during 30 min in air contaminated with 58 ppm of styrene. In our experiments an arterial blood level of more than 40 ppm was necessary before positional nystagmus could be elicited. A comparison of experiments in rabbits and in man is of course very speculative. The ethyl alcohol concentration that provokes positional nystagmus in man is, for example, about 10 times lower than the level which is nec-

essary for eliciting the same symptom in rabbits (8). The incidence of positional nystagmus following inhalation of styrene by man must thus be investigated before any conclusions about the possibility of a direct application for measurement of neurological toxicity can be drawn.

The effects of solvents on the CNS have previously been investigated with electroencephalography and psychophysiological function tests (11, 20). For investigating the basic effect of solvents on the central vestibular system, the rabbit experimental model seems suitable. Direct recordings of nerve potentials from known parts of the central vestibular system appear to be an obvious next step in the investigations (10).

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