Interaction of salicylate and noise results in mortality of rats

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Summary. Survival as a function of salicylate dose and the intensity of environmental noise was investigated in 150 adult female pigmented rats. Rats were assigned to groups (n = 6/group) defined by combinations of salicylate levels from 0- (saline) to 300 mg/kg, injected subcutaneously, and noise levels from ambient noise to 98 dB SPL, presented daily for 10-h periods for up to 17 days. Mortality occurred in groups exposed to the higher combinations of salicylate and noise.
Key words. Salicylate; noise; mortality; weight; tinnitus; rats.

Acetylsalicylic acid (aspirin) is widely used for symptomatic relief of headaches, muscle discomfort, arthritis, and more recently has been extensively advertised as a strategy for decreasing the chance of heart attack. There are reports suggesting the possibility of an interaction between salicylate and noise, which results in the enhancement of temporary or permanent shifts of the threshold of hearing[1, 2]. During experiments contributing toward an animal model of tinnitus[3], salicylate or saline was injected subcutaneously (s.c.) daily for a week in over 200 rats exposed to continuous, around-the-clock noise of 62 dB SPL (evaluated for frequency band of 1–16 kHz) for periods of 5–6 days. Very low mortality rates were observed among the rats used in our experimental paradigm but, interestingly, death occurred only in rats injected with salicylate, never in control subjects injected with saline. Since serious side effects from salicylate might have profound implications for human populations, due to the enormous popularity of aspirin taken frequently by people commonly exposed to noise, the possible toxic effects of salicylate interacting with noise were pursued. Since acetylsalicylic acid is rapidly converted in an organism to salicylic acid with a half-life of 20 min, and administration of the salt of salicylate induces analogous effects[4, 5], sodium salicylate was the drug of choice for our investigations.

Materials and methods

Female hooded rats derived from Charles River strains and bred at the University of Massachusetts at Boston, 100–120 days old, weighing 262.2 ± 3.2 g (mean ± SEM, n = 138), were housed individually for the duration of the experiment in standard laboratory cages (Fenco Products, 43 × 25 × 17 cm). Three sides of each cage were made of solid stainless steel, and one long side facing out from the housing rack was made of stainless steel wire mesh which supported a food storage bin and a water bottle. Individual cages were stored in one of two 1.5 × 2 m racks accommodating 5 rows of 3 cages. Each rack was housed in a 3.5 × 4 m acoustically shielded cubicle separate from the main rat colony. Food and water were available ad libitum, and subjects were maintained on a 12 h light/dark cycle throughout the experiment.

Background noise levels were provided by a Hewlett Packard Precision Noise Generator (Model 8057 A) and delivered through a Motorola Speaker (P 35-VAH) placed on a table located 1 m in front of the center of the animal storage rack. Sound pressure levels, recorded near the center of the storage rack, were measured by a Bruel-Kjaer Precision Sound Level Meter (Type 2203) at 55 (ambient background level), 62, 87, 95 and 101 dBA, and were approximately the same for all cages. At any given time, several groups of animals with different doses of salicylate but with the same noise level were used and distributed randomly within a block of cages. Since hearing threshold curves of rats differ significantly from those of humans, rats having higher thresholds at low frequency regions and lower thresholds for high frequencies[6], SPL noise levels were recalculated from levels determined for frequencies of 1–16 kHz in 1/3 octave bands (Quest Electronics, Impulse Precision Sound Level Meter, Model 155, and 1/3 Octave Band Filter, Model OB-133) according to standard procedures[7]. The calculated levels were 0, 44, 87, 93 and 98 dB SPL for 55 (ambient noise), 62, 87, 95 and 101 dBA SPL, respectively. The noise presented to subjects at all intensities exhibited relatively flat frequency spectra up to 20 kHz. As expected, the difference between the two methods of noise measurements appeared for low noise levels in which the low frequency ambient component, inaudible for rats, had an impact on dBA readings but was negligible for higher noise levels. In the remaining text, these noise levels will be referred to as 0, 44, 87, 93 and 98 dB SPL. The noise was presented for a daily period of 10 h, from 08.00 h until 18.00 h.

Background noise levels were combined with daily s.c. injections of sodium salicylate at doses of 350, 260, 175, and 90 mg/kg dissolved in saline, corresponding to 300, 225, 150 and 75 mg/kg of salicylic acid, and saline only. For this report, salicylic acid levels will be used to describe a given group rather than sodium salicylate doses. All subjects were weighed daily between 08.00 and 08.30 h and injected with the salicylate or saline immediately

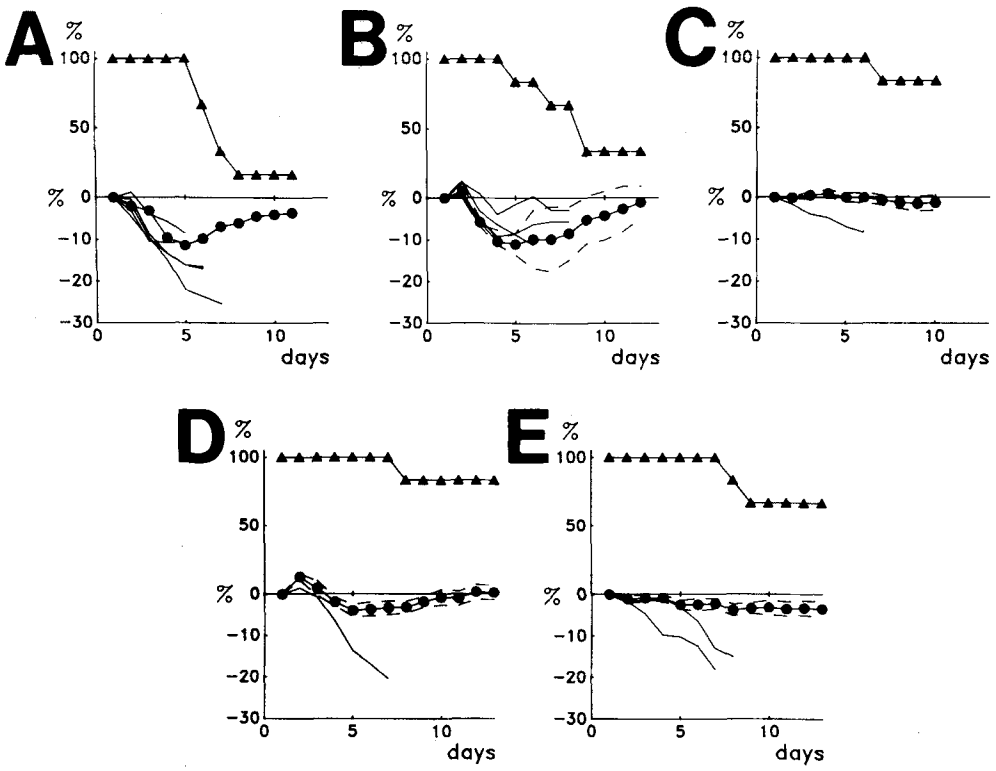
afterward, dose being dependent on the actual weight. This schedule continued for up to 17 days. Gross pathological examinations were conducted on subjects that expired during the experiment, and blood samples were obtained from part of the subjects after the experiment was completed to evaluate serum salicylate levels[8]. An additional 12 rats were used for detailed pathological examination. Daily body weights were analyzed by means of analyses of variance for partially correlated data[9], followed by Duncan tests[10].

Results

A systematic parametric study was initially performed to determine whether the interaction of different doses of salicylate with various levels of noise could act synergistically to the detriment of the organism. 20 groups of rats (6 per group) were exposed to all possible salicylate or saline and noise combinations of 4 levels of noise (0, 44, 87 and 98 dB SPL) and 5 levels of salicylate (0 [saline], 75, 150, 225 and 300 mg/kg).

The overall results of this experiment were striking. Five of the 6 subjects in the group receiving the highest salicylate dose level (300 mg/kg) and exposed to daily 10-h intervals of the loudest noise (98 dB SPL) died within 7 days of the start of the experiment, with the earliest death occurring on day 5 (fig., A). Weight loss before expiration for these subjects ranged from 8% to 25.4% of pre-experimental weight. Mortality was observed as well within other noise groups receiving the highest salicylate dose injections. Four of the 6 subjects exposed to 87 dB SPL noise died, with the first subject expiring on day 4, the second on day 6, and the last two on day 8 (fig., B). One subject died after six days in the group injected with 300 mg/kg of salicylate and exposed to 44 dB SPL (fig., C). None of the subjects died in the group injected with the highest dose level and exposed to ambient background noise only. The only death occurring outside the highest dose level was found after 7 days in the next highest dose level, 225 mg/kg, in the group exposed to the loudest noise (fig., D). The remaining 109 subjects survived.

In all cases of mortality, subjects exhibited 3–25% weight loss before expiring (fig.). Statistical evaluation of the minimal body weight of surviving animals revealed that neither salicylate alone (groups with ambient noise) nor noise alone (groups with saline) had an influence on weight changes, while the interaction of salicylate and noise resulted in highly significant weight loss (noise × salicylate interaction: $F(12,100) = 3.53$, $p < 0.001$,



Survival ratio and weight changes for groups in which mortality was observed. The upper part of each panel (triangles) represents the percent of surviving subjects out of the initial group (n = 6); the lower panels show weight changes for surviving rats (circles and dashed line representing mean ± SEM) and for expired subjects (continuous line without symbols), presented as percent of weight on day 1. *A* Salicylate 300 mg/kg, noise 98 dB SPL, initial weight (all subjects of 240.4 ± 4.4 g) (mean ± SEM); *B* salicylate 300 mg/kg, noise 87 dB SPL, initial weight of 245.7 ± 8.4 g; *C* salicylate 300 mg/kg, noise 44 dB SPL, initial weight of 227.3 ± 2.0 g; *D* salicylate 225 mg/kg, noise 98 dB SPL, initial weight of 264.2 ± 10.1 g; *E* salicylate 300 mg/kg, noise 93 dB SPL, initial weight of 267.3 ± 11.1 g.

ANOVA and Duncan test). In the group exposed to the highest combination of salicylate and noise, the body weight of surviving animals exhibited transient decrease, with the greater loss occurring around day 5 and gradual recovery (fig., A and B). Interestingly, as evident in B, surviving animals in this group had greater body weight loss than those that died, arguing against the assumption that body weight loss is directly responsible for the subject's death.

Since mortality is quite a deleterious effect and because of the relevance of our study to human health, it was decided to repeat the experiment on the 3 groups of animals exposed to a different level of noise (93 dB SPL), interposed between the higher intensities used initially. Additionally, blood samples were taken from the tails of a portion of surviving subjects on day 6, 2–2.5 h after injection, and the serum analyzed for salicylate level[8]. Two subjects in the high dose/high noise group died within 6 days (fig., E), while all of the remaining subjects survived. Statistical analysis of weight loss across experimental days revealed a lack of weight changes for surviving animals, analogous to those described in the initial experiment. Analysis of salicylate blood serum levels revealed following levels: 58.2 ± 3.2 mg/dl (mean $\pm$ SEM, $n = 4$) and 37.6 ± 2.4 mg/dl ($n = 6$) for groups 300 mg/kg, and 150 mg/kg, respectively. These means are consistent with expectations based upon previously described time-dependent uptake of salicylate, whereas for animals receiving a dose of 300 mg/kg only, the level after 2 and 2.5 h was 60.95 and 57.3 mg/dl, respectively, with the observation that there was no accumulation of salicylate during repetitive, daily injections[11]. Therefore, the salicylate-noise interaction resulting in body weight loss and even death cannot be related to abnormally high salicylate uptake in those groups.

Although we did general post mortem examinations of the subjects that had expired, we did not find any gross pathological abnormalities that could obviously account for the instances of mortality. All expiring rats appeared to have normal food and water uptake and died during the night, when they were not exposed to salicylate and/or noise. However, we sought more detailed pathological analyses to determine the physiological basis for mortality. Twelve additional rats underwent the same procedure as described previously divided into three groups: 1) noise intensity at 98 dB SPL and 350 mg/kg sodium salicylate injection; 2) 98 dB SPL noise exposure with injections of saline; 3) injections of 350 mg/kg with no additional noise presented.

Two of the subjects in the high noise/high salicylate group died on the night of day 5, just prior to the intended time of evaluation on day 6. The surviving 2 subjects from that group and 2 subjects each randomly selected from the other groups were submitted for complete pathological examination.

Histopathological examination of lungs, cerebrum, cerebellum, bone marrow, salivary glands, cervical lymph nodes, esophagus, trachea, thyroid, ovaries, uterus, vagina, left and right auricle, left and right ventricle, I.V. septum, liver, kidney, tongue, stomach, duodenum, pancreas, jejunum, ileum, cecum, colon and rectum revealed neither changes that might be related to mortality nor dissimilarities permitting differentiation of subjects belonging to specific groups. There was no evidence of bleeding from the gastrointestinal tract nor from any other organs analyzed, and the hemograms from each rat examined did not indicate that they suffered form any internal blood loss. All blood parameters were within normal limits.

Discussion

The main finding of this experiment is that while the interaction of salicylate and noise resulted in mortality, with weight decreases usually preceding the instances of mortality, neither salicylate nor noise alone had an impact on mortality or weight change. It is important to stress that mortality occurred systematically in three temporally separated experimental series, excluding the possibility of random, nonspecific causes of the mortality. Additionally, the results suggest parametric dependence of mortality rate on salicylate/noise levels, further strengthening our results. At this stage, it is difficult to speculate on the possible physiological mechanisms of our observations. The fact that salicylate or noise alone was without marked influence seems to underscore that only the interaction of these variables produces harmful effects.

It is premature at this stage to speculate on the physiological basis of the mortality observed, except perhaps the indication that general stress is involved. From this point of view, assumption of salicylate-induced tinnitus producing stress in animals might be of interest. Results obtained from our animal model of tinnitus[3], which use salicylate and the Pavlovian suppression paradigm of learned appetitive reaction, support this possibility. Interestingly, the pathological evaluations precluded internal gastrointestinal bleeding or bleeding within any other organ as the cause of death, which might be one of the first possibilities suspected in the case of salicylate toxicity.

While it is difficult to relate our observations directly to humans, several analogies can nevertheless be made. First, it is worth pointing out that the levels and duration of noise exposure used in this experiment are within the parameters to which people are exposed during work. 10 h of exposure per day correlates approximately with the normal time of day that workers are subjected to higher sound levels than at home. The noise levels used presently correspond in range from those of a very quiet office environment to those in close proximity of heavy equipment in a factory. Although the highest dose of salicylate exceeded doses prescribed routinely in humans, serum salicylate levels were close to those measured in humans

treated for rheumatic arthritis [12]. Furthermore, it is impossible to exclude the likelihood of harmful effects other than mortality which we were not able to detect, as indicated for example, by temporary weight loss in surviving animals. Thus, there is a possibility of unnoticed harmful impact of the interaction of salicylate and noise in humans.

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GABA-A-mediated gastrin release induced by baclofen in the isolated vascularly perfused rat stomach

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Summary. In order to investigate the role of peripheral GABA-B receptors, the effects of the putative GABA-B agonist baclofen on immunoreactive gastrin release from an isolated vascularly perfused rat stomach preparation were examined. The vascular infusion of baclofen at graded concentrations induced a dose-dependent increase in gastrin release; this was unaffected by the GABA-B antagonist delta-aminovaleric acid, but was fully prevented by the selective GABA-A antagonist bicuculline as well as by atropine or tetrodotoxin. These results suggest that the stimulant effects of baclofen are mediated by nervous cholinergic structures associated with GABA-A receptors, and indicate that this GABA-B agonist must be regarded as a partial agonist of peripheral GABA-A receptors.
Key words. Gastrin release; rat isolated stomach; baclofen; GABA-A receptors.

Several lines of evidence indicate that GABA-A receptors participate in the regulation of gastric acid secretion. Experiments in vivo in anesthetized rats have demonstrated that activation of central GABA-A receptors is associated with a marked dose-dependent and bicuculline-sensitive increase in acid secretion [1,2].
Experiments in vitro on isolated guinea pig stomach have shown that the GABA-A receptor agonist muscimol induces a dose-dependent enhancement of acid secretion which is antagonized by bicuculline, but the putative GABA-B receptor agonist baclofen does not. This indicates that peripheral GABA-A, but not GABA-B receptors, participate in the regulation of acid secretion [3]. Moreover, in rat isolated gastric antral mucosal fragments, GABA produced a dose-dependent bicuculine- and atropine-sensitive stimulation of gastrin release, probably mediated by the activation of antral cholinergic neurons associated with GABA-A receptors [4].
Whether or not peripheral GABA-B receptors are involved in the regulation of gastrin release remains unknown. The purpose of the present study was to investigate the effects of baclofen on gastrin release from the isolated vascularly perfused rat stomach.

Methods

Experiments were carried out on male Sprague-Dawley rats weighing 200–220 g, 24 h fasted, but with free access to water.
Isolated vascularly perfused rat stomach. The isolation of the stomach was performed using the technique described by Saffouri et al. [5] and Martindale et al. [6], with minor modifications. The animals were anesthetized with