tion (0.04 mg/ml) of SQ_{20881} used in vitro approximates the concentration present in the blood following the i.v. injection of 0.4 mg of this inhibitor. Kininase activity was also inhibited in plasma and lung when lead acetate was added during the incubation (table 1).

Kininase II activity was also measured in plasma and lung from rats which had previously been injected with SQ_{20881} or lead acetate. Rats were killed at 5 min and 60 min post-injection and the results are shown in table 2. In this case, injected lead acetate does not inhibit kininase activity in plasma and lung. The nonapeptide, SQ_{20881}, markedly reduces kininase activity in the plasma during the first 5 min after injection and even 60 min post-injection only 30% of the control activity was detected in the plasma. In lung, however, SQ_{20881} administered in vivo did not alter kininase activity at either time period.

Additional experiments were performed to determine the effect of SQ_{20881} on the endotoxin lethality. Rats were given various doses of *S. marcescens* endotoxin, and SQ_{20881} or phosphate buffered saline. The 0.4 mg dose of SQ_{20881} used in vivo has been shown to be effective in potentiating the action of bradykinin in the rat[9]. Mortality was observed after 72 h and the results in table 3 show that the kininase inhibitor had no appreciable effect on endotoxin sensitivity. In subsequent experiments, SQ_{20881} was given 1.5 h before, and simultaneously with endotoxin. Again there was no alteration in lethality due to the SQ_{20881}. In an additional group of animals, bradykinin was injected along with the kininase inhibitor and endotoxin (table 4). Even under these conditions with very high circulating bradykinin these was no increase in endotoxin morbidity.

This work confirms the results of previous investigators[10,11] that the nonapeptide, SQ_{20881} when added in vitro is a very effective inhibitor of kininase II activity in rat plasma and lung. Furthermore, the in vitro addition of lead acetate was shown to inhibit kininase activity in plasma and lung. The concentration of lead acetate used in vitro approximates the amount present in the blood immediately following the injection of 10 mg of lead acetate in a 200 g rat. This dose of lead acetate markedly sensitizes rats to small quantities of endotoxin[12]. It was clear, however, from the lethality data presented in this study that SQ_{20881} did not sensitize rats to endotoxin. Even the combination of kininase inhibitor and bradykinin did not effect endotoxin morbidity. These results lead to the conclusion that marked alterations in level of circulating kininase activity do not play a critical role in the lethal effects of endotoxin and that the mechanism of lead sensitization is not due to its kininase inhibiting properties.

9 A. Bianchi, D. B. Evans, M. Cobb, M. T. Peschka, T. R. Schaeffer and R. J. Laffan, Eur. J. Pharmac. *23*, 90 (1973).

10 R. Igic, E. G. Erdos, J. Yeh, K. Sorrells and T. Nakajima, Circulation Res. *30/31*, Suppl. II, 51 (1972).

11 S. Oparil, T. Koerner and J. K. O'Donoghue, Circulation Res. *34*, 19 (1974).

12 H. Selye, B. Tuchweber and L. Bertok, J. Bacteriol. *91*, 884 (1966).

Offset-induced audiogenic seizures[1]

Chia-Shong Chen and G. R. Gates[2]

Department of Psychology, Monash University, Clayton, Victoria 3168 (Australia), 21 March 1977

Summary. A relatively stereotyped seizure reaction can be triggered by the 'offset' of an intense bell sound in C57BL/6J mice. Susceptibility to this offset-induced audiogenic seizure was found to depend upon the age of the animals tested (higher in older mice) and the duration of the noise exposure (more effective with longer exposure).

When certain strains of mice are exposed to intense noise, they may show a characteristic stereotyped sequential reaction consisting of wild running, clonic seizure and in some cases tonic seizure and death. This phenomenon is widely known as audiogenic seizure. Susceptibility to audiogenic seizure may be due to the genetic background of the animals: highly seizure-prone mice are likely to seize on the first exposure to an intense noise whereas normally-resistant mice are not[3]. However, seizure susceptibility can be induced in seizure-resistant strains of mice by drug-withdrawal procedures (e.g. ethanol[4,5] or barbiturate[6] withdrawal) or by a priming procedure which consists of exposing animals to an intense noise a few days prior to testing for audiogenic seizure[7,8]. In this report we describe a different type of audiogenic seizure reaction which, to the best of our knowledge, has not been documented in the literature. The classic audiogenic seizure phenomenon described presviously refers to a seizure driven by continuous acoustic stimulation. The seizure to be described here is, instead, triggered by the offset of intense auditory stimulation.

Animals used in this experiment were C57BL/6J mice which are normally regarded as being seizure-resistant[3]. However, they can be made seizure susceptible by priming at certain ages[7]. The offset-induced audiogenic seizure was discovered when we were testing for the rate of spontaneous audiogenic seizure (i.e. animals who seize on the very first exposure to noise) in mature C57BL/6J mice. Animals of various ages were exposed to a 125–127 dB (re 0.0002 dyn/cm^2) bell sound for 60 sec or until seizure occurred. In the process of testing we noted that some animals showed a relatively stereotyped motor display after the bell was turned off. Typically, an animal would suddenly jerk backwards into an exaggerated standing posture with all 4 limbs rigidly extended. The whole body shook vigorously and the tail writhed in a lateral, reptile-like swimming motion. Before collapsing into a relatively relaxed and flattened posture, most of

1 This work was supported by the Australian Research Grants Committee fund to the first author.

2 Current address: Department of Psychology, University of Melbourne, Melbourne, Australia.

3 J. L. Fuller and F. R. Sjursen, J. Hered. *58*, 135 (1967).

4 J. L. Fuller and R. E. Wimer, in: Biology of the Laboratory Mouse, p. 609. Ed. E. L. Green. McGraw-Hill, New York 1966.

5 G. Freund and D. W. Walker, Psychopharmacologia, Berlin *22*, 45 (1971).

6 G. R. Gates and C.-S. Chen, Nature *249*, 162 (1974).

7 K. R. Henry, Science *158*, 938 (1967).

8 C.-S. Chen and G. R. Gates, Medikon *4*, 19 (1975).

the animals would swing their bodies sideways and 'bob' their heads up and down a few times. This 'offset' seizure usually occurred immediately after the offset of the bell sound and lasted from 3 to 10 sec. Delayed reactions were noted in only 3 instances and latencies for these were estimated at 0.5–1 sec. Those animals which exhibited these offset seizures also showed a characteristic behavioral pattern during bell stimulation. This consisted of an initial startle response to the loud sound followed by movement about the test chamber for a short time (usally about 10 sec). The animals would then freeze into a tense, motionless posture for the remainder of the exposure except for occasional head movements.

The incidence of offset-induced seizure appears to be a function of the age of the animals tested. The figure, a, shows the percentage of mice showing offset seizure when they were tested at either 40, 100, 160 ± 5, 200 ± 5, 300 ± 5 or 400 ± 5 days of age. The results indicate that young animals rarely show this type of seizure; the percentage of seizure was highest for the 2 middle age groups and declined when the animals grew older.

In addition to the age factor, the incidence of seizure response is also dependent on the duration of the noise exposure. 1 week after first testing for offset seizure, mice of the 200-day-old group were repeatedly tested every second day under 4 different exposure durations (5, 15, 30 and 60 sec). The order of testing for each condition was randomized for each mouse. The results as shown in the figure, b, indicate that the offset seizure does depend on the duration of the noise exposure (Cochran's Q-test: $Q[3] = 16.03$, $p < 0.01$). A 5-sec-exposure was not very effective in inducing offset seizure as compared with longer exposure durations. There is also an indication that longer noise exposure may result in longer offset seizure. Mean duration of the seizure response for the 5-, 15-, 30- and 60-sec-groups were 3.4, 4.9, 5.2 and 5.7 sec, respectively. Overall comparison of the 4 groups (based on results of 3 mice that showed offset seizure on every test) indicate that the difference was significant at the 0.075 level (Friedman's 2-way-analyisis of variance $\chi^2_r = 6.6$); the difference among the latency scores of the 15-, the 30- and the 60-sec-group (based on results of those 7 mice that seized on each test) was significant at the 0.052 level ($\chi^2_r = 6.33$). The difference between the 30-sec- and the 60-sec-group was not significant (Wilcoxon's matched pairs test, $T = 22$).

Susceptibility to offset seizure appears to remain stable over a long period of time. 3 months after testing for the effect of exposure duration, all 14 mice (one died) were again tested in a random order under each of 3 exposure durations: 60, 90 and 120 sec. In order to reduce the sequential effect of repeated noise exposure, the second test was conducted 4 days after the first and the 3rd test 6 days after the 2nd test. The results, as shown in the figure, b, indicate little change in incidence of seizure over the 3-month-period (under the 60-sec-condition only 2 mice failed to seize on the 2nd test). It is of interest to note that the incidence of offset seizure was higher in this experiment than that shown by animals of comparable age (300 days old) in the previous experiment (figure, a). This could be due to the fact that these animals were not naive to offset seizure. They had been made to seize at a susceptible age. It suggests that experiencing seizure at a sensitive period may make the seizure risk 'permanent'. No significant change in seizure rate and duration of seizure was noted for the 3 exposure durations.

Intense sensory stimulation may induce florid seizures in both animals and man[9,10]. However, the results of this experiment demonstrate, in a rather striking fashion, that offset of sensory stimulation (in this case auditory stimulation) may also act as an potent trigger for seizure precipitation.

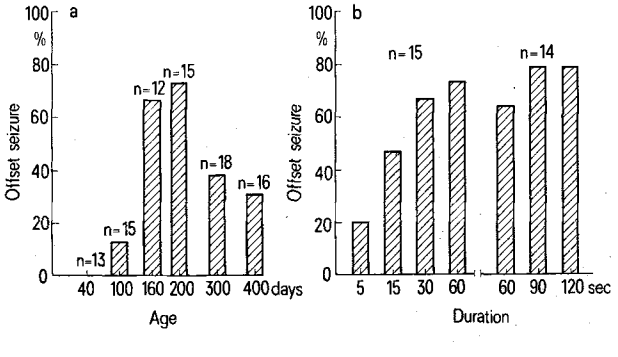
It is not clear how these offset seizures are induced. However, there are a few interesting features which may provide clues as to the mechanism(s) involved.

1. The fact that offset seizure risk is age-dependent suggests that the phenomenon may be related to the maturational state of nervous system: The younger animals, which rarely showed offset seizure generally did not maintain the motionless, frozen posture shown by the older, offset seizure-prone mice during the noise exposure. This finding implies that their nervous systems were reacting to the noise in a different fashion.

2. The offset seizure reaction is 'audiogenic' in nature because it only occurred at the offset of the bell sound and after a certain minimum duration of acoustic stimulation (see the figure, b).

3. The fact that incidence of seizure seems to increase with duration of exposure implies that offset seizure depends upon a build-up of stimulation-induced neural activity which is released by stimulus offset. That offset of stimulation may act as an effective stimulus for triggering seizure may not seem so surprising in view of physiological data which show that there are cells in the auditory pathway which respond to offset of stimulation[11,12]. In fact the present result raises the question of whether stimulus duration may affect the size of such offset responses.

4. The fact that offset seizures have not been observed in mature BALB/c mice[13] suggests that there may be a genetic basis for offset seizure risk. Finally, it is of interest to note that chlorpromazine (1 mg/kg) sufficient to suppress priming induced 'onset' audiogenic seizure in this strain of mice has no effect on offset seizures incidence (unpublished data). Thus different neural mechanisms may be involved in modulation of 'onset'-induced and 'offset'-induced audiogenic seizures. This may provide a useful model for exploring the nature of inhibitory function in the central nervous system.



9 W. Bevan, Psychol. Bull. *52*, 473 (1955).
10 F. M. Forster, in: Physiological Effects of Noise, p. 151. Ed. B. L. Welch and A. S. Welch. Plenum Press, New York–London 1970.
11 G. R. Bock, W. R. Webster and L. M. Aitkin, J. Neurophysiol. *35*, 265 (1972).
12 L. M. Aitkin and S. M. Prain, J. Neurophysiol. *37*, 512 (1974).
13 G. R. Gates and C.-S. Chen, Exp. Neurol. *47*, 360 (1975).