EFFECTS OF CHRONIC ADMINISTRATION OF KANAMYCIN ON CONDITIONED SUPPRESSION TO AUDITORY STIMULUS IN RATS

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Abstract—The conditioned suppression technique was employed to study the ototoxic effects of chronic administration of the antibiotic, kanamycin. Lever pressing behavior for food reinforcement of rats was suppressed in the presence of an auditory stimulus (sound) or visual stimulus (light) that had been previously paired with electric shocks. Repeated administration of kanamycin at the dose of 400 mg/kg/day for more than 50 days significantly attenuated the conditioned suppression to auditory stimulus but did not attenuate the conditioned suppression to visual stimulus. This finding suggests that the attenuating effect of chronic administration of kanamycin on conditioned suppression to auditory stimulus can be interpreted in terms of the selective action of the drug on the auditory system.

The ototoxicity and nephrotoxicity of aminoglycoside antibiotics, i.e., streptomycin, kanamycin, neomycin, gentamycin and aminosidine, and polypeptide antibiotics, i.e., viomycin, capreomycin and tuberactinomycin are well known. For testing ototoxicity caused by these antibiotics in animals, methods based on pinna reflex (1, 2), cochlear microphonies (3, 4, 5) have been reported. The pinna reflex test is easily carried out but not sensitive enough to detect hearing loss. In addition, subjective observations play a considerable role and the validity of the results is questionable. Methods based on cochlear microphonics involve difficulties in implanting chronic electrodes on the round window of small animals such as mice or rats.

There are excellent operant procedures for detecting hearing loss caused by antibiotics using titration methods (6, 7). Although these methods have advantages over the others mentioned above, a great deal of training is necessary to stabilize their response under a titration reinforcement schedule. Therefore, the application of a simpler reinforcement schedule was required for drug screening tests.

In the conditioned suppression or the conditioned emotional response (8), lever pressing behavior for food reinforcement is suppressed in the presence of a previously neutral stimulus that had been accompanied repeatedly by an electric shock. Although the conditioned

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suppression procedure is frequently used for testing anti-anxiety drugs (9), the important aspect of the stimulus control in this behavior is the testing of the degree of sensitivity of the sensory system.

The purpose of the present study was to investigate the effects of chronic administration of kanamycin on conditioned suppression using auditory stimulus and also to evaluate the applicability of the operant technique in detecting drug ototoxicity.

MATERIALS AND METHODS

Subjects

Eight adult, male Sprague-Dawley rats, all experimentally naive, were used. They were maintained at approximately 80% of their free-feeding body weights throughout the experiment, and were kept in individual cages in which they had free access to water.

Apparatus

An operant conditioning chamber (24 x 21 x 31.5 cm) was placed in a sound attenuating cubicle (40 x 40 x 75 cm). The chamber had a lever which required a minimum force of approximately 20 g to operate. A pellet feeder (Ralph Gerbrands) located behind the front wall of the chamber dispensed 45-mg pellets into a hopper. A stimulus light (100v, AC) was mounted above the lever. Auditory stimuli were 4K or 10 KHz interrupted pure tones produced by an oscillator (Nagashima Medical Instruments, Model PA). The tones lasted 1 sec, followed by 2 sec of silence, and trangents were not audible by the human ear. The speaker (Pioneer, Model PT-7) was located 30 cm above the top of the plexiglass roof of the chamber which had 25 holes (5 mm diameter each). The intensity of the tone was calibrated by a Bruel and Kjaer sound level meter placed so that the condensor microphone faced upwards in the middle of the operant conditioning chamber. 3 mA of electric shock was delivered to a grid floor of the chamber via a shoker. Experimental contingencies and data recording were arranged by a PDP 8/I computer (Digital Equipment). Cumulative response recorders (Ralph Gerbrands) were also used and all such equipment was located in the adjoining room.

Procedure

Rats were assigned arbitrarily to three groups. Three rats in group 1 were used to test the effect of chronic administration of kanamycin on conditioned suppression using sound as the conditioned stimulus. Three rats in group 2 were used to test the effect of eardrum lesion under the same training condition given group 1. Two rats in group 3 were used to test the effect of chronic administration of kanamycin on conditioned suppression using light as the conditioned stimulus. In the first stage of training, all rats in the three groups were kept under a random ratio schedule in which a random number of lever pressings on the average of 20 times was reinforced with a food pellet. After stabilization of the responses under this schedule, the second stage of training was begun. A light serving as a conditioned stimulus was turned on for one min, every 4 min under the random ratio schedule. During this conditioned stimulus period, electric shock was delivered for 0.5 sec. once or twice at
random series. The shock delivery was independent of the lever pressing. After several pairings of light and shocks, the presence of the conditioned stimulus alone was sufficient to inhibit lever pressing for positive reinforcement. By repeated training under this conditioned suppression schedule, no response in the light period and a relatively high rate of response in the other periods (nonconditioned stimulus period) were observed. In the third stage of training, rats in group 1 and group 2 were kept under the conditioned suppression schedule using tones instead of light, while the other conditions were kept the same as those in the second stage of training. 4K and 10 KHz pure tones were used as conditioned stimuli in random order in a session. The intensity of the tone was 70 decibels (dB) in sound pressure level (SPL) for 4K and 10 KHz as a rule and was varied when necessary.

After conditioned suppression to the tones was established, the training ended. The rats in group 1 were then given kanamycin sulfate (Meiji, Kanamycin sulfate) i.m. at the dose of 200 mg/kg twice daily. Although the injections were given every day, except Sunday, the rats were tested for one hour once a week under the conditioned suppression schedule using sound, and were also tested under the conditioned suppression schedule using light, at the end of the chronic administration of kanamycin.

Rats in group 2 had bilateral lesions in the eardrums after the third stage of training, and were tested under the conditioned suppression schedule using the tones on the days after the lesions.

Rats in group 3 continued to be trained under the same schedule as that in the second stage of training. After conditioned suppression to light was established for these rats, injections of kanamycin sulfate and tests were given in the same manner as those for rats in group 1, except that light was used as the conditioned stimulus instead of the tones.

The data analysis was based on the response rates in the conditioned stimulus periods and non-conditioned stimulus periods in each session. The suppression ratio (S. R.) was defined as follows:

\[
\text{S.R.} = \frac{B - A}{B}
\]

A: response rate in the conditioned stimulus (CS) period.
B: response rate in the non-conditioned stimulus period.

Suppression ratios were averaged for all pairings of CS and non-CS periods for each rat in each one hour test session, and averaged for the rats in the same group of the same experimental conditions. The averaged suppression ratio in each session was compared with that in pre-drug or pre-lesion sessions by using the t-test.

RESULTS

Repeated training under the conditioned suppression schedule with 4K and 10 KHz tones produced clear conditioned suppression of lever pressing behavior for food reinforcement, as shown in a sample cumulative response record in Fig. 1. During the CS period, the animals did not press the lever, and instead showed gross behavioral changes such as freezing and exploration. A sample cumulative response record in Fig. 2 shows that repeated
FIG. 1. Sample cumulative response records illustrating conditioned suppression to auditory conditioned stimulus (CS). The event pen on the bottom line shows the presentation of auditory CS (4K or 10 KHz tone). The pip of the event pen indicates the delivery of electric shock (UCS). The pip of the response pen indicates the delivery of a food pellet.

FIG. 2. Sample cumulative response records illustrating the attenuation of conditioned suppression to auditory CS in a rat given daily injections of kanamycin (400 mg/kg/day) for 56 days. Note the high rate of response during the CS period. Other details as in Fig. 1.

injections of kanamycin for more than 50 days did not suppress response during the auditory CS period. Neither freezing nor exploration was observed during this period. In Figs. 3 and 4, the mean suppression ratio for the 4K and 10 KHz tones, and the mean response rate in non-CS periods before either tone are shown for 3 rats in group 1. Daily injections of kanamycin for 41 days did not affect conditioned suppression to the auditory stimuli of 4 KHz at 70 or 60 dB (SPL) and of 10 KHz at 70 or 60 dB (SPL). However, after 52 daily injections of kanamycin, a significant decrease (P<0.05) in the mean suppression ratio was observed with 4 KHz at 60 dB (SPL) and 10 KHz at 50 dB (SPL). The ratios did not recover significantly to the control level with repeated injections of kanamycin, despite the fact that the intensity of the tones was increased. However, a significant increase was observed for the suppression ratio by replacing the auditory CS with the visual one during the final session. The mean response rate in the non-CS periods was gradually decreased by repeated injections of kanamycin.

In order to compare the hearing loss by chronic administration of kanamycin with
Fig. 3. Effect of chronic administration of kanamycin on the mean suppression ratio for 4 KHz CS and mean response rate in non-CS periods before the presentation of 4 KHz tone in each session. Each value indicates the mean standard error (SE) of 3 rats. The intensity of the tone in decibels (sound pressure level) is presented at the top. "C" refers to the control test in which the experimental conditions were the same as those in the following test except that no drug was given before the test.

Fig. 4. Effect of chronic administration of kanamycin on the mean suppression ratio for 10 KHz CS and the mean response rate in non-CS periods before the presentation of the 10 KHz tone in each session. Other details as in Fig. 3.
that by eardrum lesion, the effects of the eardrum lesions on conditioned suppression to auditory CS were tested. The mean suppression ratio for 4K and 10 KHz tones and the mean response rate in the non-CS periods before either tone are presented in Fig. 5.

**Fig. 5.** Effect of bilateral eardrum lesion on the mean suppression ratio for 4K or 10 KHz CS and the mean response rate in non-CS periods before the presentation of each CS. Each value indicates the mean ± SE of 3 rats. The intensity of CS tone was 70 dB (SPL) with 4K and 10 KHz. “C” refers to the control test in which the experimental conditions were the same as those in the following tests except that eardrums were not injured.

**Fig. 6.** Effect of chronic administration of kanamycin on the mean suppression ratio for visual CS (light) and the mean response rate in the non-CS period. Each value indicates the mean ± SE of 2 rats. “C” refers to the control test in which the experimental conditions were the same as those in the following tests involving chronic administration of kanamycin, except that no drug was given before the test.
intensity of the tones was 70 dB (SPL) with 4K and 10 KHz. The suppression ratio for the 10 KHz tone decreased from 0.77 during the pre-lesion test referred to as “C” in Fig. 5 to 0.51 after the eardrum lesions, in both ears, and the mean response rate for the non-CS periods before the 10 KHz tone also decreased by about 40%. A similar tendency was observed with the 4 KHz tone. The statistical test showed that the decrease in the mean response rate in the non-CS periods before the 4K or 10 KHz tone was significant (P<0.05), while the decrease in the suppression ratio for the 4K or 10 KHz tone was not significant.

The conditioned suppression to visual CS was not markedly affected by chronic administration of kanamycin in the present experiment using 2 rats in group 3, as shown in Fig. 6. The mean suppression ratio was always above 0.76, although mean response rate in the non-CS period decreased gradually after 35 days of kanamycin injections.

DISCUSSION

Repeated intramuscular injections of kanamycin for 108 days did not increase response suppressed by visual CS. On the contrary, conditioned suppression to auditory CS was significantly attenuated after 52 daily injections of kanamycin. Furthermore, the reduced suppression ratio during auditory CS recovered to the pre-drug level by replacing the auditory CS with the visual CS. These findings suggest that kanamycin attenuates conditioned suppression in a different mode of action from that of anti-anxiety drugs, which are postulated to attenuate conditioned suppression as a result of selective action on central mechanisms concerned with emotional states (10). This reduction of the response suppression in the conditioned suppression paradigm to auditory CS seems likely to be responsible for the hearing impairment caused by kanamycin.

There was no marked difference between the 4K and 10 KHz tones used in the present experiments in onset of reduction of the suppression ratio. This finding differs from those of recent studies on ototoxicosis due to antibiotics in which it was demonstrated that hearing loss occurs first in high frequency tones and then progresses toward low frequency tones (11, 12, 13, 14). The gradual decrease in mean response rate in the non-CS period that was seen following about 30 daily injections of kanamycin may be attributed to ataxia due to daily injections into the quadriceps femoris muscle in the hind limb and to a nephrotoxicity indicated by hematuria.

The decrease in suppression ratio observed after eardrum lesion was less than that produced by repeated injections of kanamycin. A similar decrease in response rate during non-CS periods suggests surgical damage as a result of the eardrum lesion. On the other hand, the gradual increase in the suppression ratio indicates a steady recovery from and adaptation to the hearing impairment. Lorente de No’ and Harris (15) reported that animals without eardrums showed a hearing loss of not more than 20 to 30 dB. It is well known that when the eardrums are missing, hearing occurs by air-conduction directly to the round window and by bone-conduction through the skull around the middle ear to the inner one. Perhaps conduction by these routes may produce no statistically significant decrease in the suppression ratio after eardrum lesion while the marked decrease by kanamycin may be due
to the irreversible toxic effect on the auditory system. The fact that the decreased suppression ratio after eardrum lesion recovered to the control level in a day or two suggests a rapid regeneration of the eardrum in the rat.

In conclusion, it is suggested that the attenuating effects of kanamycin on conditioned suppression to auditory CS can be interpreted in terms of selective action on the auditory system, and that an operant technique is useful in detecting ototoxicity of antibiotics.

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