Stimulus conditions influencing the development of tolerance to repeated cold exposure in rats

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Tolerance to an environmental cold challenge in rats is eliminated when cold exposure occurs in a context different from the adaptation context, indicating that learning mechanisms play a role in thermoregulation (Riccio, MacArdy, & Kissinger, 1991). This finding, analogous to outcomes obtained with drug tolerance, was investigated in the present study. Experiment 1 demonstrated that a change in both proximal and distal contextual cues disrupts an established cold adaptation, an outcome consistent with the view that associative processes contribute to the tolerance. In Experiment 2, although cold tolerance persisted over a 7-day retention interval, the disruption of tolerance by a shift in context was attenuated with the delay of testing. This finding suggests that the precise stimulus attributes of the context were forgotten over the interval. Experiment 3 demonstrated that cold-tolerance disruption is due to the actual change in context and not to novelty of the test context. Experiment 4 showed that changing the context associated with each cold exposure impaired the development of tolerance. The results of these experiments provide additional evidence that cold tolerance is regulated at least partially by associative learning processes.

There is growing evidence that a purely pharmacological explanation of drug tolerance is inadequate (Siegel, 1975, 1976, 1977, 1988, 1989; Tiffany & Maude-Griffin, 1988). Drug adaptation systems are modulated by a number of nonpharmacological events, including conditioning manipulations. For instance, drug tolerance is disrupted when an animal is tested in a context that differs from the one in which drug adaptation has occurred. Such control by contextual cues is robust for a variety of drugs, including morphine, (Siegel, 1975, 1976, 1977), ethanol (Melchior & Tabakoff, 1981), and scopolamine (Poulos & Hinson, 1984).

To account for this effect of contextual stimuli, Siegel (1975) proposed an interpretation of drug tolerance based on Pavlovian conditioning. According to this account, the drug serves as the unconditional stimulus (US) and the adaptation context functions as a conditional stimulus (CS+). Unlike in a traditional Pavlovian analysis, however, the CS is assumed to elicit a response that

is opposite to the physiological reaction to the drug following pairings of the two stimuli. This conditioned compensatory response (CCR) attenuates the effect of the drug by counteracting the unconditioned response, thereby producing drug tolerance. Furthermore, testing a drug-tolerant animal under cuing conditions that differ from those of training removes the source of the CCR and results in a loss of drug tolerance (see Siegel, 1989, for a review). Although a habituation explanation that does not invoke a CCR has been proposed (Baker & Tiffany, 1985), it too emphasizes the critical role of contextual cues in drug tolerance.

Similar compensatory or habituating mechanisms also appear to play a role in the development of tolerance to environmental stressors (Fregly, 1953; Riccio & Campbell, 1966; Riccio, MacArdy, & Kissinger, 1991). For instance, rats immersed briefly in cold water show a large decline in body temperature on the first exposure. Repeated exposure to the stressor over 7 consecutive days results in cold tolerance such that progressively smaller declines in, and faster recoveries to, normal body temperature are observed with each successive exposure. However, when adapted rats are exposed to cold in a context consisting of different distal and proximal cues, tolerance is largely abolished, with body temperature decreasing to the level obtained during the first exposure session. This contextual specificity suggests that associative processes influence the physiological mechanisms of thermoregulation and the development of cold adaptation (cf. Poulos & Cappell, 1991).

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The purpose of the present set of experiments was to investigate the effect of contextual cues on the adaptation to a cold stressor by determining which attributes of the adaptation context gain control over cold tolerance, and whether repeated changes in the context influence the rate of adaptation. In addition, we attempted to determine whether the attributes of a context are forgotten over a retention interval and whether the disruption of cold tolerance, which occurs with a change in context, is independent of the novelty of the test context.

EXPERIMENT 1

Riccio et al. (1991) established that contextual stimuli play a role in adaptation to cold exposure in rats. In that study, the context being manipulated consisted of a variety of cues such as the room where testing occurred and a restraining device. Although a context by its very nature represents a global, multidimensional stimulus situation, these cues can be viewed as consisting of two general classes: *proximal* cues associated with the restraining device, and *distal* cues, such as the characteristics of the room in which cold exposure occurs. This distinction between contextual characteristics has received little attention. The purpose of the present experiment was to assess the relative control over cold tolerance exerted by proximal cues, distal cues, and their combination, as indicated by the disruption of cold adaptation when these elements are changed.

In Experiment 1, cold adaptation was examined under four conditions. Rats in all conditions were subjected to a brief cold water immersion on each of five consecutive days. We expected this regimen to result in a smaller decline in body temperature on the 5th day than on the 1st day of cold exposure (cf. Hamm, Knisely, & Lyons, 1990; Kokkinidis, 1986; Riccio & Campbell, 1966; Riccio et al., 1991). On the test day (Day 6), one group was exposed to the cold in a different restraining tube and different room from those used during adaptation, so that both proximal and distal cues were changed in the test. A second group experienced a shift in only proximal cues (the restraining tube) on the test day, and a third group experienced a shift in only distal cues (the room). A fourth, control group was exposed to the cold in the same context as had been used during adaptation. Each condition was counterbalanced with respect to room and restraining tube.

Method

Subjects. Forty experimentally naive male Long-Evans rats (90–120 days of age) were purchased from Charles River, Wilmington, MA. The rats were housed individually in hanging wire-mesh cages in a room maintained on a 16:8-h light:dark cycle. Food and water were freely available throughout the experiment. All subjects were handled for 4 min each day, beginning 2 days prior to experimental treatment.

Apparatus. A Model 2095 Forma Temp Jr water bath (Marietta, OH) maintained at 4°–5°C was used to lower the rats' body temperatures. Colonic body temperatures were measured by a digital thermometer (Fisher Scientific, Pittsburgh, PA). Clear or black

Plexiglas tubes used to restrain rats during water immersion served as proximal cues. Both the clear and the black tubes were constructed of cylindrical Plexiglas, with numerous holes to allow water circulation.

Different rooms were used for training and testing and served as distal cues. One room (Context A) consisted of bright fluorescent illumination and the constant sound of several operating water bath pumps. The second distal context (Context B) consisted of a smaller room adjacent to the animal colony that was dimly illuminated by an over-head fluorescent light, contained the noise of only a single water bath, and was permeated by rat colony odors. Thus, Contexts A and B differed in location and transport cues, size, brightness, sounds, and odors.

Procedure. Rats were assigned randomly to one of four groups of 10 rats each. On the 1st day of cold stress, we exposed all the rats to cold by partial immersion up to their necks in cold water and obtained the duration of exposure required to lower each rat's body temperature to $21 \pm 1^\circ\text{C}$. Body temperatures were measured immediately following each immersion by inserting the temperature probe 1.5 cm into a rat's rectum. This exposure time for each subject was then maintained on succeeding days. Thus, all rats had their body temperatures lowered to the same point on Day 1 and were then immersed for a constant duration on the remaining days, including the test day.

On Days 1–5, half of the rats ($n = 20$) were immersed in cold water in clear tubes in Context A; the other half of the rats ($n = 20$) were adapted in black tubes in Context B. This procedure was used to assess and control for any possible differences in construction or characteristics of the restraining tubes.

Testing occurred on Day 6 under one of four conditions. Five rats from each of the conditions above ($n = 10$) were tested under the same (S) proximal (Prox) and distal (Dist) cues as during training (Group S–Prox/S–Dist). A second group was exposed to cold in different (D) proximal (Prox) and distal (Dist) cues than were used during adaptation (Group D–Prox/D–Dist) such that 5 rats from each training condition were shifted from a clear (or black) restraining device in Context A (or B) to a black (or clear) restraining device in Context B (or A) in the test. A third group (D–Prox/S–Dist) was tested under a shift in only the proximal restraining device, and a fourth group (S–Prox/D–Dist) was tested under a shift in distal room conditions. This $2 \times 2 \times 6$ factorial design allowed both between- and within-group assessments of the contextual control of tolerance to cold water.

Results and Discussion

Mean body temperatures for the four conditions across training days and the test are presented in Figure 1. Mean immersion times, which were required to lower body temperature to the criterion 21°C on the first exposure session, did not differ among groups ($F < 1$: S–Dist/S–Prox = 661.8, D–Dist/D–Prox = 682.8, S–Dist/D–Prox = 675.0, and D–Dist/S–Prox = 657.0 sec). All groups showed a similar rise in mean body temperature over successive exposure Days 2–5. Body temperature during testing (Day 6) varied with context conditions. Animals tested with different proximal cues and same or different distal cues (Groups D–Prox/S–Dist and D–Prox/D–Dist) showed a disruption of tolerance on Day 6. Tolerance was not disrupted, however, in rats tested with the same proximal cues and either the same or different distal cues (Groups S–Prox/S–Dist, S–Prox/D–Dist).

A mixed-design analysis of variance (ANOVA: group $\times$ day) on body temperature on Days 1–5 yielded a significant main effect of day [$F(4,128) = 112.01, p < .05$].

Figure 1. Mean rectal body temperatures of rats following brief cold water immersion. Contextual stimuli remained constant for all subjects on Days 1–5. Warmer body temperatures on Day 5 reflect adaptation to cold. On Day 6, Group D-Prox/D-Dist was exposed to the cold treatment in a context consisting of different proximal and distal cues. Group D-Prox/S-Dist was exposed to the cold in a context consisting of different proximal cues only, and Group S-Prox/D-Dist was exposed to the cold in a context differing only in distal cues. Group S-Prox/S-Dist remained in the original context.

Furthermore, cold tolerance was disrupted by changing the context in which the animal experienced the cold adaptation. The contexts in which the rats were adapted (clear tube/Context A versus black tube/Context B) did not produce a difference in adaptation and test, and therefore the counterbalanced conditions were combined. Another mixed-design ANOVA (group $\times$ day) yielded a significant interaction when the test day was included in the analyses [$F(15,180) = 11.79, p < .05$], indicating that the contextual change resulted in a significant disruption of body temperature on Day 6. Dunn tests for between-group comparisons indicated that on Day 6 all rats receiving cold exposure in a different context (D-Prox/D-Dist, S-Prox/D-Dist, and D-Prox/S-Dist) differed significantly from the temperatures of animals immersed in the same context on Day 6 (S-Prox/S-Dist) (all $ps < .05$). In addition, body temperatures of animals receiving exposure to different distal cues only (S-Prox/D-Dist) differed significantly from temperatures of the animals receiving different proximal and different distal cues (D-Prox/D-Dist) and different proximal cues only (D-Prox/S-Dist) ($p < .05$). Furthermore, Dunn tests for within-group comparisons indicated that on Day 6 the body temperatures of the S-Prox/S-Dist group remained the same as on the previous day, while the temperatures of the remaining groups decreased significantly (all $ps < .05$).

The present findings provide further evidence of context specificity in adaptation to cold exposure in rats. As expected, the daily brief cold exposures produced adaptation, as measured by the reduction in the severity of body temperature decrease. Furthermore, consistent with the findings in Riccio et al.'s (1991) study, a change in both proximal and distal contextual cues produced a

profound attenuation of the tolerance: body temperatures following cold exposure in the altered context were close to those on the 1st day of exposure. Interestingly, a change in only the proximal cues (the restraining tube) was sufficient to produce a tolerance disruption equal to that of a change in both proximal and distal cues. A change in only the distal cues (room characteristics) also produced a disruption of tolerance, but it was not nearly as large in magnitude as that caused by altering the proximal cues or proximal and distal cues together. The characteristics of the restraining tube apparently are more salient to the animal because the tubes provide more associative control over cold adaptation than do the characteristics of the room.

EXPERIMENT 2

The evidence of associative control over cold tolerance suggests that other learning principles also should influence the expression of tolerance. For example, the well-established flattening of a stimulus generalization gradient over time implies that a change in stimulus conditions should be less effective in disrupting cold tolerance after a retention interval (Perkins & Weyant, 1958; Thomas & Lopez, 1962; for a review, see Riccio, Richardson, & Ebner, 1984). Such an outcome has been reported previously for morphine-induced drug tolerance (Feinberg & Riccio, 1990). When tolerant rats were tested in a different context 2 days after their last drug exposure, they showed partial disruption of tolerance: the analgesic effect of morphine returned, a finding consistent with that obtained in many other studies (see Siegel, 1989, for a review). However, when a 7-day interval was interpolated between the final tolerance conditioning exposure and testing, the shift in context conditions failed to reduce tolerance. Thus, drug tolerance itself was retained well, but stimulus control over the tolerance was diminished. The aim of Experiment 2 was to determine whether a similar outcome occurs for the adaptation to cold exposure.

Method

Subjects. Thirty-two adult male Long-Evans rats served as subjects. Twenty-two of the subjects were experimentally naive, and the remaining 10 rats were subjects from the S-Prox/S-Dist condition of Experiment 1. All the rats were 90–120 days old and were housed and handled exactly as were those in Experiment 1. To control for the possible confound of weight gain during the 7-day retention interval, food deprivation for each animal was introduced during the interval to maintain the animals' body weights at approximately their mean weights during the 6-day training phase. All the rats were weighed daily to assess the change in body weights.

Apparatus. The apparatus was the same as in Experiment 1.

Procedure. We adapted all the rats to the cold water according to the same procedure as that in Experiment 1. Because the present experiment was conducted simultaneously with Experiment 1, the S-Prox/S-Dist rats from Experiment 1 also served as subjects in the present experiment. These animals, along with an additional 22 rats, were exposed to the cold in a clear tube/Context A or in a black tube/Context B on Days 1–6.

Following adaptation, the rats were randomly assigned to one of four treatment conditions. In the first condition (Same-Long), rats

were tested in the same context following a retention interval of 7 days. This condition was to determine whether tolerance remained following the 1-week retention period during which no cold exposure occurred. In the second condition (Diff-Long), each rat was tested in a context consisting of different proximal and distal cues following the 7-day retention interval. On the test day (Day 13), each animal was again immersed in the cold water in the appropriate context for the duration provided on Day 1. Rats in the third condition (Diff-Short) were tested in the different context on Day 7 and thus had a 24-h retention interval. This condition served as a context shift control. A fourth condition (Same-Short) served as a same context control; each of these rats was tested on Day 7 in the same context as that used during adaptation. Body temperatures were recorded upon the animals' removal from the cold water on each immersion day.

Results and Discussion

Mean immersion times, which were required to lower body temperatures to the criterion 21°C on the first exposure session in Groups Same-Long (661.8 sec), Diff-Long (711.0 sec), Diff-Short (620.0 sec), and Same-Short (665.0 sec), did not differ significantly on the 1st day of adaptation [$F(3,36) = 3.24, p > .05$].

Mean body temperatures on test day for the four conditions are presented in Figure 2. At the short (1-day) interval following the last training session, rats tested in the different context showed a loss of tolerance (Group Diff-Short), whereas rats tested in the same context showed tolerance (Group Same-Short). Moreover, rats tested in the same context after the long (7-day) retention interval remained adapted to the context (Group Same-Long). The change in context following the long interval was still effective in disrupting tolerance in Group Diff-Long.

A mixed-design ANOVA (group $\times$ day) indicated that groups did not differ significantly in mean body temperatures during adaptation days. However, all groups showed a significant increase in body temperatures across these days [$F(5,140) = 115.59, p < .05$], indicat-

ing that cold tolerance developed over Days 1–6. When data from the test day were included in this analysis, a significant group $\times$ day interaction occurred [$F(18,168) = 8.44, p < .05$], indicating that the context shift on the test day disrupted tolerance. Dunn tests ($\alpha = .05$) revealed that Day 7 (the short retention test day) temperatures were significantly lower than were those of Day 6 in Group Diff-Short. Likewise, following the long (7-day) retention interval, body temperatures in Group Diff-Long decreased significantly relative to those on Day 6. Analysis of the test day data in a 2×2 ANOVA (context condition $\times$ retention interval) yielded a significant main effect of context condition [$F(1,28) = 43.74, p < .05$]. The interaction between variables was not statistically significant. Nonetheless, Dunn tests revealed that temperatures in Group Diff-Long were significantly higher during testing than were those in Group Diff-Short, suggesting that the extent of disruption displayed in the long-delay condition was weaker than was that in the short-delay test.

These results indicate that tolerance to cold remains over a 7-day period of no exposure. Furthermore, the disruption of tolerance following a shift in context was attenuated when testing occurred 7 days after adaptation. This outcome is consistent with the view that the forgetting of stimulus attributes reduces the magnitude of the context-shift effect (Feinberg & Riccio, 1990; Riccio et al., 1984).

EXPERIMENT 3

Experiments 1 and 2 demonstrate that a shift in contextual cues disrupts tolerance to a cold stressor. However, whether the impairment reflects the absence of critical stimuli associated with cold exposure, or a disruptive effect due to the novelty of the altered cues, is not clear (see Thomas & Empedocles, 1992).

One procedure that can be used to prevent the contribution of novelty to test effects is to adapt subjects in one context and then use the animal colony room as the test context. In this case, the altered context is not novel but highly familiar to the subject. Using this procedure, Siegel (1976) and Siegel, Hinson, Krank, and McCully (1982) found that tolerance was still disrupted when animals were tested in their home (familiar) environment, suggesting that drug tolerance was disrupted by the change in context and not merely by the novelty of the test context. In a study of morphine tolerance, using a discrimination procedure, Hinson and Siegel (1986) administered a drug to animals in one context on alternate days and saline injections in a different context on intervening days. The contexts differed in characteristics such as light, odors, and so forth. Following tolerance development, the animals were tested in the nondrug administration (S-) context. The animals displayed a loss of tolerance in the now equally familiar context that had not been associated with the drug.

The purpose of Experiment 3 was to assess the effects of familiarity with the testing context on disruption of

Figure 2. Mean rectal body temperatures of rats following brief cold water immersion on test days occurring after a short or long interval after adaptation. Groups Diff-Short and Diff-Long were exposed to the cold treatment in contexts differing in both proximal and distal cues, 1 day or 7 days, respectively, following adaptation. Groups Same-Short and Same-Long were exposed to the cold in the original context 1 day or 7 days following adaptation.

the tolerance to cold. Using an approach similar to that of Hinson and Siegel (1986), we exposed animals to the cold water in one context (CS+) and, on alternate days, to a different context (CS-) which was not paired with a cold water exposure. Thus, the alternate context (CS-) predicted the absence of the cold exposure (US). Animals adapted to the cold in a clear tube/Context A were familiarized with the altered context of black tube/Context B without immersion into the cold water on days following administration of cold immersion. Following five exposures to each context, the experimental subjects were exposed to the cold water in the now familiar context of black tube/Context B.

Method

Subjects and Apparatus. Ten experimentally naive male Long-Evans rats (90–120 days of age) were housed and handled as in Experiment 1. The apparatus used in the present experiment was the same as that used in Experiment 1.

Procedure. Because the results of Experiments 1 and 2 showed that tolerance to cold does not differ on the basis of context in which the adaptation develops, and because other experiments limited the availability of the equipment, the present experiment was not counterbalanced with respect to contexts.

All animals were adapted to cold in the CS+ context (clear tube/Context A) in the same manner as in Experiment 1. Exposure to cold, however, occurred on Days 1, 3, 5, 6, and 9. On Days 2, 4, 6, 8, and 10, the animals were restrained in a black tube/Context B (CS- context) and suspended into an empty water tank for the duration determined on Day 1. On Day 11, half ($n = 5$) of the rats were immersed in cold water in the black tube/Context B (CS-). The other rats were exposed to cold in the training tube and context (CS+). Body temperatures were measured daily upon the animals' removal from either context.

Results and Discussion

Figure 3 shows mean body temperatures in each cold exposure group during adaptation and the test day (Day 11). Rats that received the daily cold exposures on alternate days prior to the context shift demonstrated adaptation to the cold. Furthermore, the body temperature of rats tested on Day 11 in the same context did not

differ from their body temperature on the final adaptation day (Day 9). However, rats tested in a context different from that used during adaptation showed a large reduction in body temperature relative to that during the last adaptation session.

A mixed-design ANOVA (group $\times$ day) indicated that body temperatures increased significantly across Days 1–9 [$F(4,32) = 102.51, p < .05$]. Furthermore, Dunn tests ($\alpha = .05$) for within-group analyses revealed that the body temperature of animals tested on Day 11 in the same context as prior exposures did not differ from their body temperature on the final adaptation day (Day 9). However, animals that received the cold exposure on Day 11 in the previous CS- context (Diff) had a significant reduction in body temperature from that on Day 9. A Dunn test for the between-group comparison indicated that on Day 11 the animals exposed to cold in the CS- context differed significantly from the animals immersed in the CS+ context.

The differential conditioning procedure used here afforded equal exposure to both the training and test contexts during the acquisition phase of the experiment. Given that changing the context on the test day disrupted cold tolerance, even though the new context was familiar to the subjects, novelty of the altered context at testing cannot be a necessary condition for disrupting tolerance. Thus, as Hinson and Siegel (1986) found with morphine tolerance, the loss of cold adaptation resulting from an altered test context must be a result of the removal of contextual retrieval cues. The results of this experiment provide further evidence that tolerance to cold is mediated by an associative mechanism.

EXPERIMENT 4

Experiments 1–3 indicate that context plays an important role in the development of adaptation to environmental stressors such as cold. In these experiments, as in many studies of drug tolerance, context remained constant during adaptation, and its associative role was revealed when a context shift at testing reduced tolerance. An interesting but apparently unexplored strategy for examining associative processes in tolerance involves context shifts during the training exposures. Epstein, Caggiula, Perkins, McKenzie, and Smith (1991) conducted such a study, in which human subjects repeatedly smoked nicotine cigarettes either in the same context or in different contexts on each exposure session. Epstein et al. found that subjects who smoked in the constant context developed nicotine tolerance, as indicated by decreased heart rate. However, subjects exposed to the nicotine in multiple contexts did not develop the tolerance. With respect to the present concerns, these data suggest that altering the context at the time of each exposure to cold should disrupt the development of tolerance, or at least weaken it. If a context becomes a signal for cold and elicits a compensatory response in preparation for cold stress, then preventing a particular context from being reliably associated with the cold ex-

Figure 3. Mean rectal body temperature of rats receiving brief cold exposure on Days 1, 3, 5, 7, and 9. On Days 2, 4, 6, 8, and 10, all subjects were exposed to a context consisting of altered proximal and distal cues. On Day 11, Group Diff received the cold treatment in the context used for the even numbered days, while the Group Same received the cold treatment in the original adaptation context.

posure should eliminate or attenuate the development of tolerance.

Experiment 4 examined the development of cold tolerance when the cold exposures were administered each day in a different context. One group of rats received daily exposures to cold in the same restraining tube for 4 days (a reliable tolerance can be developed in a 4-day period). A second group also was exposed to the cold water for 4 days; however, different restraining tubes were used for each immersion. If the development of tolerance depends on a reliable signal for cold exposure, then rats experiencing cold exposure in different contexts should show less adaptation to cold, as indicated by a greater reduction in body temperature on Day 4 relative to the group exposed to cold in the same context.

Method

Subjects and Apparatus. Twelve adult male Long-Evans rats 90–120 days of age served as subjects. Six of the animals were experimentally naive, and the remaining 6 also served as control animals in Experiment 2. Again, these experiments were conducted simultaneously in order to permit use of a common control condition and more efficient use of animals. All subjects were housed and handled in the same way as were those in the previous experiments.

The same equipment as in the previous experiments was used, with the addition of the following restraining tubes: a white tube similar in construction to the clear and black tubes, and a wire mesh tube constructed of hardware cloth. Thus, the four restraining tubes were clear, black, white, or wire.

Procedure. The basic design of the present experiment consisted of two groups. Group Daily-Diff ($n = 6$) was exposed to the cold water each day in a different tube. Exposure time was based on the time required to reduce body temperature to 21°C on Day 1, regardless of tube type. The order of exposure to the tubes was counterbalanced across days within Group Daily-Diff, with the exception that on Day 4 all rats were restrained and immersed in the clear tube. Use of the same tube for both groups on the final exposure day controlled for any possible differences among the restraining tubes. The control group (Daily-Same, $n = 6$) were the same animals as those used in the Same-Short and Diff-Short conditions from Experiment 2, which were immersed on each day in the clear restraining tubes. Because the two experiments were conducted simultaneously, the data from the first 4 days of adaptation in those animals from Experiment 2 were used for the comparison group in this experiment. Upon the animals' removal from the tubes, their body temperatures were recorded.

Results and Discussion

One animal in Group Daily-Diff died during the adaptation procedure and was not replaced. Therefore, Group Daily-Diff contained 5 rats.

Figure 4 shows mean body temperatures in each of the two groups over the four cold exposure sessions. Rats exposed to cold water in the same context on each day adapted to the cold (Group Daily-Same). By contrast, rats receiving the daily cold exposures in different contexts (Group Daily-Diff) showed only slight adaptation. A mixed-design ANOVA (group $\times$ day) yielded a significant main effect of day [$F(3,27) = 27.51, p < .05$] and a significant group $\times$ day interaction [$F(3,27) = 12.48, p < .05$]. A Dunn test on Day 4 data indicated that the body temperatures in Group Daily-Diff were signif-

Figure 4. Mean rectal body temperature for rats receiving brief cold water immersion in either the same context for 4 days (daily-same) or a different context for each of the 4 days (daily-diff).

icantly less than were the body temperatures in Group Daily-Same ($p < .05$). That some tolerance developed in Group Daily-Diff group, however, is reflected in the significant difference ($p < .05$) of a within-subjects comparison between the first and last exposure days.

These data indicate that the associative control of cold tolerance begins at the first exposure to the cold, given that a context shift on the second exposure was sufficient to disrupt the development of cold tolerance. It is apparent that continually changing the context at each cold exposure continues to prevent normal tolerance development. However, as Figure 4 shows, there is a slight increase in body temperatures across days in Group Daily-Diff, suggesting either that there is some generalization across the different contexts or that some degree of tolerance may be independent of context.

It is possible that the repeated shifts in context masked or attenuated the expression of tolerance rather than its development. If this were the case, one would expect to see the rapid acquisition of tolerance in a transfer task. Thus, if, following the fourth context shift, the stimuli were held constant for several subsequent cold exposures, any latent learning should be reflected in a relatively rapid increase in tolerance. To examine this possibility, the rats in Group Daily-Diff were given 2 additional days of cold exposure in clear tubes. Thus, these animals received a total of 3 days' exposure in the same restraining tube following the prior exposures in different contexts and their tolerance was compared with the original 3-day adaptation of the animals in Group Daily-Same.

These additional exposure sessions resulted in a rate of tolerance development that was identical to that shown by Group Daily-Same during their first 3 days of exposure. The mean body temperature on the 3rd day of exposure to the same context in the transfer test in Group Daily-Diff was 24.4°C; Group Daily-Same had a mean body temperature of 24.7°C on their 3rd day of original cold exposure. A mixed-design ANOVA of these

data yielded significant main effect of day [$F(2,18) = 44.27, p < .05$], indicating that both groups developed tolerance during their first 3 days of exposure to cold in the same context. The main effect of group was not statistically significant, suggesting that the effects of cold exposure in the altered contexts had little impact on the later development of adaptation in the context. Therefore, the results of this additional manipulation suggest that the relative lack of tolerance with constantly changing contexts reflects the absence of an association (cue with cold exposure) rather than an impairment of expression of cold adaptation.

GENERAL DISCUSSION

The findings of the present experiments provide further evidence that associative processes mediate adaptation to acute cold exposure in rats. In four experiments, the daily brief cold water exposures produced tolerance as measured by the reduction in the severity of hypothermia resulting from each exposure; however, in each case, an alteration of environmental context was sufficient to reduce the resistance to cold.

Altering proximal or distal contextual characteristics, or both (Experiment 1), revealed that the proximal cues gain more associative control over adaptation to cold than do distal stimuli. Rats tested in a different restraining tube either in the same room or in a different room showed more severe tolerance disruption than did animals tested with altered distal cues only. Although the proximal cues of the restraining tube are probably particularly salient to the animal, the distal cues of the context also appear to acquire associative value. A shift in distal cues also resulted in the attenuation of cold adaptation, although less so than did the change in the proximal context.

In Experiment 2, we examined the effects of a relatively long (7-day) interval placed between the adaptation phase and the test. In addition to demonstrating the persistence of tolerance in the same-context condition, the findings provided evidence that a contextual change on the test day resulted in the disruption of cold adaptation following the delay interval. However, changing the context 7 days after adaptation produced a weaker disruption than that which occurred for the short (24-h) retention interval. This finding is consistent with the less severe generalization decrement with delayed testing that has been reported for both animals and humans (Riccio et al., 1984), and it resembles the outcome of a similarly designed morphine tolerance study (Feinberg & Riccio, 1990).

That the disruption of established cold tolerance was not due to the novelty of the test context but to the change in the test context was shown in Experiment 3. Animals that had been familiarized with the test context through repeated prior exposure in a discrimination paradigm still displayed a disruption of tolerance. Although Experiment 3 did not contain a condition in which animals were adapted to one context and tested in an unfamiliar

context, the severity of disruption of tolerance in the animals tested in the altered but familiar context appeared very similar to that of the disruptions of tolerance found in animals tested in unfamiliar contexts in Experiments 1 and 2. The results of Experiment 3 were similar to those obtained with morphine tolerance in a similar (Hinson & Siegel, 1986) paradigm and indicate that the loss of tolerance cannot be attributed to mere novelty of the test context.

If tolerance depends on the development of associative linkages between contextual stimuli and cold exposure, then a design in which a different context accompanies each exposure should retard the development of tolerance (see also Epstein et al., 1991). Such an outcome was obtained in Experiment 4. The lack of tolerance with changing contexts contrasts with the tolerance that results when cold exposures are administered in the same context. Furthermore, the contextual cues begin to gain associative control of the tolerance development at the initial pairing of context and cold water immersion.

The outcomes of Experiments 1–4 fit quite well with the classical conditioning explanation of morphine tolerance proposed by Siegel and his colleagues. In that model, the conditioned response in the drug tolerance situation is antagonistic or compensatory to the effect of the drug. This compensatory response balances the response to the drug and the net result is observed as an attenuated drug effect. Several studies have shown that similar compensatory processes may be involved in the conditioning of the immune system (Dyck, Osachuck, & Greenberg, 1989; Frank & MacQueen, 1988). It seems possible that a similar mediating compensatory mechanism is responsible for tolerance to thermoregulatory challenge. For example, the cold stressor (US) causes a reduction of the animal's body temperature (UR). The daily cold exposures include restraining tube and room cues that serve as the contextual CS. Initially, the CS cues alone have no effect on the organism; however, after being paired with the cold water US, the contextual stimuli presumably begin to activate physiological responses that prepare the animal for the cold, resulting in better maintenance of body temperature (tolerance). With respect to the drug studies, when testing occurs in a different context, the CS cues are not present and the preparatory response is not engaged. As a result, rats respond to the drug much as they do on the initial presentation. Similarly, when cold-tolerant subjects are tested in a different context, the CS is no longer available to elicit compensatory responses to the challenge, and adaptation to cold is not expressed. Although in the present study we did not attempt to assess directly the presence of a compensatory conditioned response, recent data from our laboratory suggests that such a mechanism exists (Dobis, Morgan, & Riccio, 1993). Since a compensatory conditioned response to cold stress is likely to involve increased heat production and/or activation of mechanisms to reduce heat loss, we assumed that cold-tolerant rats might be momentarily impaired if given a

heat challenge. Accordingly, cold-tolerant rats were exposed to warm water in the presence of contextual stimuli previously associated with the cold immersions. Relative to nontolerant controls, these animals showed a transient but reliably greater elevation in body temperature. Furthermore, the hypothermic "overshoot" tended to be reduced when tolerant subjects were tested in the absence of cold-associated cues. These findings are consistent with the view that a CCR contributes to thermoregulatory tolerance.

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