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Tolerance and Cross-Tolerance to Central Nervous System Depressants After Chronic Pentobarbital or Chronic Methaqualone administration[1]

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COMMISSARIS, R L AND R H RECH *Tolerance and cross-tolerance to central nervous system depressants after chronic pentobarbital or chronic methaqualone administration* PHARMACOL BIOCHEM BEHAV **18**(3) 327–331, 1983 —This study reports on tolerance and cross-tolerance to the rotarod (RR)-disrupting effects of various central nervous system (CNS) depressants Female rats trained on the RR were fed ground chow containing pentobarbital (PB, 2 0 mg/g chow) or methaqualone (MQ, 1 0 mg/g chow) and were injected twice daily (PB) or daily MQ) with 30 mg/kg IP for 6 days Control rats received ground chow and saline injections On day 7 the subjects were tested with various doses of PB, MQ, diazepam (DZ), or ethanol (ET) for disruption of RR performance over the time-course of the drug effect (up to 12 hours) Control animals demonstrated a dose-dependent duration of impairment for all 4 agents Both groups receiving chronic drug showed a prominent decrease in duration of RR impairment after PB, a less marked decrease after MQ, and even less of a decrease after DZ However, neither chronic drug group showed an appreciable tolerance to the RR disruption of ET, relative to the control group Based on the time of 50% recovery (RR performance recovering to 90 seconds or more), both chronic treatments resulted in a significant shift of the dose-response curves for PB, MQ and DZ to the right Therefore, the degree of tolerance and cross-tolerance in rats chronically treated with PB or MQ was dramatic for PB and MQ, was significant for DZ, but was not demonstrable for ET

Tolerance Cross-tolerance Diazepam Methaqualone Pentobarbital Ethanol Depressants Rotarod

TOLERANCE is defined as a decrease in the effects produced by a drug, relative to the initial actions, after repeated administration Chronic administration of drugs of the general central nervous system (CNS) depressant class often results in tolerance to their effects and cross-tolerance to the effects of other CNS depressants of this type (see reviews [6, 7, 9]) This tolerance can be either dispositional or functional in nature The former is characterized by changes in the absorption, distribution, metabolism or excretion of the drug which result in a decreased concentration of the drug at the neuronal system involved in the drug effect Functional, or cellular tolerance, is characterized by an adaptation by which the neuronal system involved in the drug effect becomes less sensitive to a given concentration of drug [1] A number of agents of the general CNS depressant class have been reported to enhance hepatic microsomal metabolic capacity [8, 12, 15] Chronic administration of these agents would be likely to result in dispositional tolerance and cross-tolerance to the effects of other agents whose effects are terminated by these hepatic microsomal oxidation reactions Past research has indicated that dispositional tolerance to barbiturates can occur within a few days [6], but methaqualone has been little studied in this regard Furthermore, few experimental studies have attempted to assess cross-tolerance to other types of CNS depressants during this early period of chronic treatment [7, 9, 11] The majority of reports on cross-tolerance among members of this drug class appear to be based on clinical anecdotal information

The present study examined the effects of one week of chronic pentobarbital (PB) and chronic methaqualone (MQ) treatment on tolerance and cross-tolerance to the rotarod (RR)-disrupting effects of PB, MQ, diazepam (DZ) and ethanol (ET) in rats Previous studies indicated that this level of chronic PB dosing produced, for hypothermic effects of PB, a dispositional but not functional tolerance [3] Furthermore, a similar regimen of chronic PB treatment for more than three weeks yielded only marginal evidence for development of functional tolerance to the rotarod-impairing effects of PB and ET [2]

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TABLE 1
CHRONIC PENTOBARBITAL AND CHRONIC METHAQUALONE TREATMENT REGIMENS

	Chronic PB*			Chronic MQ*	
Day	Drug in Food (mg/g)	IP Injection (mg/kg) a m	p m	Drug in Food (mg/g)	IP Injection (mg/kg) a m only
1	2 0	30	—	1 0	30
2–5	2 0	30	30	1 0	30
6	0 0	30	—	0 0	30
7	0 0	Test Drug RR	—	0 0	Test Drug RR

*PB=pentobarbital, MQ=methaqualone RR=rotarod test

METHOD

Animals

The subjects were drug-naive female Sprague-Dawley rats (Spartan Farms, Haslett, MI) weighing 200–225 grams at the start of the experiment Subjects were housed three per cage in a room with a 12 hr light-dark cycle (lights on 0700–1900 hr) with water supplied ad lib and fed from ceramic food cups containing either ground chow alone or chow containing a drug supplement (see below)

Rotarod (RR) Procedure

All subjects were trained to walk the RR before the start of the experiment RR training and testing consisted of placing the subject on a narrow rotating cylinder (18 cm wide 10 5 cm diameter, 9 rpm) and measuring the time (180 seconds maximum) until the animal fell onto a padded platform three feet below [13] Subjects were considered trained or fully recovered when they could maintain themselves on the RR for 180 seconds on two consecutive trials Following training, the subjects were randomly assigned to one of the three treatment conditions described below Following chronic treatments, subgroups of each treatment category received a test dose of one of the 4 drugs studied and were then examined for duration of RR disruption RR performance was determined at 5, 10, 15 and 30 minutes post-injection following a test agent and every 30 minutes thereafter until recovery to determine the duration of drug-induced disruption Each animal was tested with only one drug dose

In addition to plotting the magnitude of RR disruption for various doses of the agents used, we also determined recovery from drug-induced disruption on the basis of the time required for the return to the 50% level of control performance [13] This value was defined as the time of the first trial post-drug during which the subject remained on the RR for 90 sec or more This measure was found to be less variable than other criteria and allowed us to better establish dose-response curves for RR disruption induced by these drugs The dose levels injected for each test drug were chosen on the basis of pilot studies, to achieve the best compromise for the lowest dose of each agent that induced mild ataxia and the highest dose that was still sub-anesthetic, being established at the time of peak drug effect

Chronic Drug Administration Procedure

Protocols for administration of chronic PB or MQ are shown in Table 1 Control rats received ground chow without drug and saline injections The doses of chronic drug treatments as described in Table 1 were based on drug effects in pilot studies The chronic PB regimen with twice-daily injections and the chronic MQ regimen with once-daily injections, plus the amounts in the food, initially produced a near-anesthetic level of depression of about the same duration The treatment regimen combining injections with oral dosing in the food was also considered to better simulate the clinical situation usually characterized by long-term intake of maintenance doses with intermittent bouts of exposure to larger doses superimposed Neither chronic drug treatment resulted in subject lethality as great as five percent

Statistics

The non-parametric Mann-Whitney U-test was used to compare the data from two groups at each of the different time intervals Dose-response curves were compared by analysis of variance In all statistical evaluations $p<0.05$ was chosen as the criterion for statistical significance [10]

Drugs

Pentobarbital sodium (PB) was purchased from Sigma Chemical Co (St Louis, MO) both IP test doses and drug concentrations in the food refer to the salt Methaqualone (MQ) was purchased from William Rorer, Inc (Fort Washington, PA), both IP test doses and drug concentrations in the food refer to the free base Diazepam (DZ) was generously supplied by Hoffmann-LaRoche (Nutley, NJ), test doses refer to the free base suspended in 0 5% methylcellulose solution (also the vehicle for methaqualone injections) Ethanol (ET) was administered at a 10% solution (v/v) in saline

RESULTS

Both chronic PB and chronic MQ administration significantly reduced the duration of RR disruption following administration of various test doses of PB (Fig 1) Similarly, the duration of RR impairment produced by various test

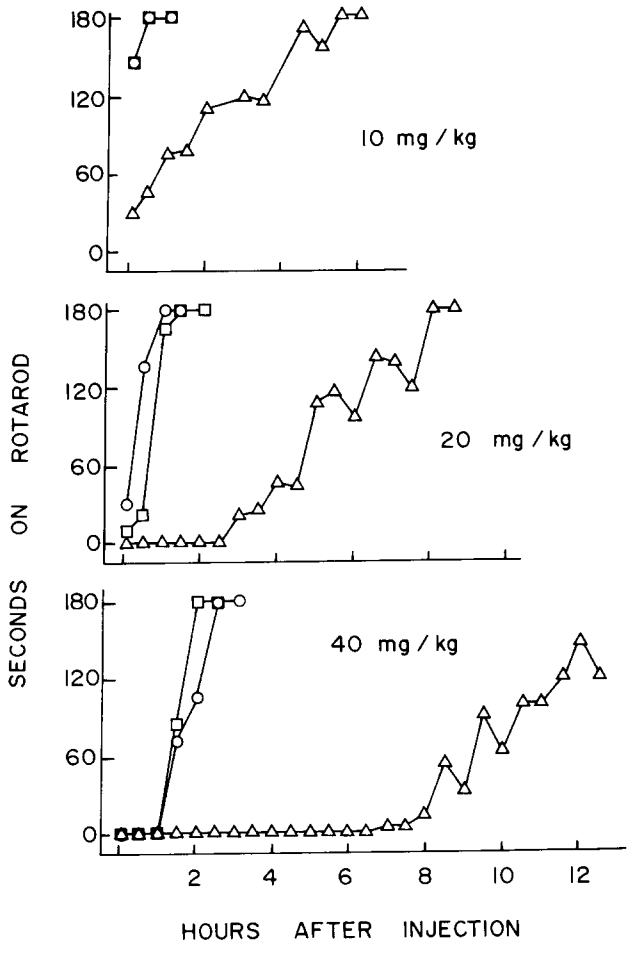

FIG 1 The time-course for the effects of PB on rotarod performance in control chronic PB-treated and chronic MQ-treated rats The duration (sec) which control (△) chronic PB-treated (○) and chronic MQ-treated (□) subjects walked on the rotarod at various intervals after injection of 10 (top panel) 20 (middle panel) and 40 (bottom panel) mg/kg PB are plotted Each point represents the mean from at least four subjects Both chronic treatments significantly attenuated the duration of rotarod impairment produced by all three doses of PB

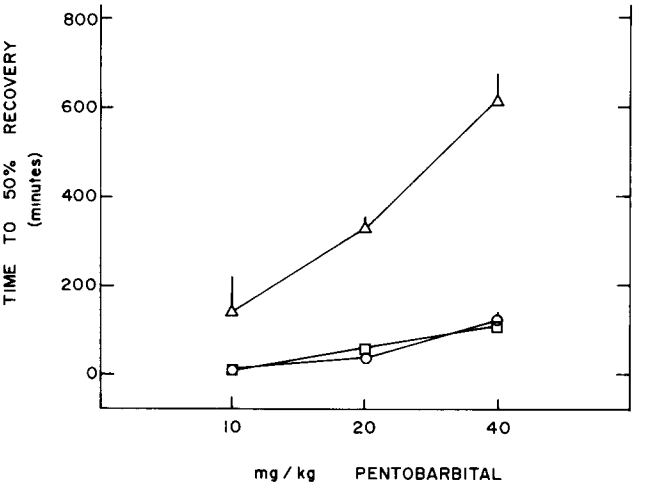

FIG 2 The dose-response effect of PB on rotarod performance in control, chronic PB-treated and chronic MQ-treated rats The times (min) to 50% recovery of rotarod performance (90 sec or greater) produced by various doses of PB are plotted for control (△), chronic PB-treated (○) and chronic MQ-treated (□) subjects Each point and vertical bar represents the mean ± S E M from at least four subjects Points without vertical bars indicate that the S E M is smaller than the diameter of the symbol Both chronic treatments significantly attenuated the effects of PB as indicated by the marked shift to the right in the dose-response curves

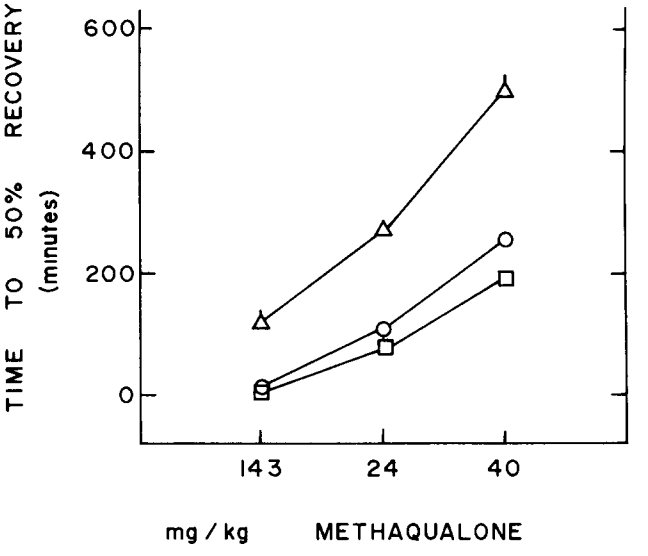

FIG 3 The dose-response effect of MQ on rotarod performance in control, chronic PB-treated and chronic MQ-treated rats The times (min) to 50% recovery of rotarod performance produced by various doses of MQ are plotted for control (△), chronic PB-treated (○) and chronic MQ-treated (□) subjects Both chronic treatments significantly attenuated the effects of MQ, as indicated by the shift to the right in the dose-response curves See Fig 2 legend for other details

doses of MQ and DZ was also significantly attenuated by both chronic PB and chronic MQ administration (not illustrated) However, there was no significant effect of either chronic PB or chronic MQ treatment on the duration of ET-induced RR impairment for any of the test doses examined

Using the time to 50% recovery criterion, both chronic PB and chronic MQ treatments resulted in a marked shift to the right in the dose-response curves for PB (Fig 2) There was no difference as to the extent of tolerance development to the PB test dose between the chronic PB and chronic MQ groups When MQ test doses were administered (fig 3), the tolerance induced to MQ in the chronic PB animals did not differ from that in the chronic MQ subjects However, the shortened duration of rotarod disruption after the MQ test doses was less marked than for the PB test doses, for both the chronic PB and chronic MQ groups When DZ was tested (Fig 4), the degree of tolerance manifested in the chronic drug-treated subjects was less prominent still There was a trend for the DZ effects to be of shorter duration in the

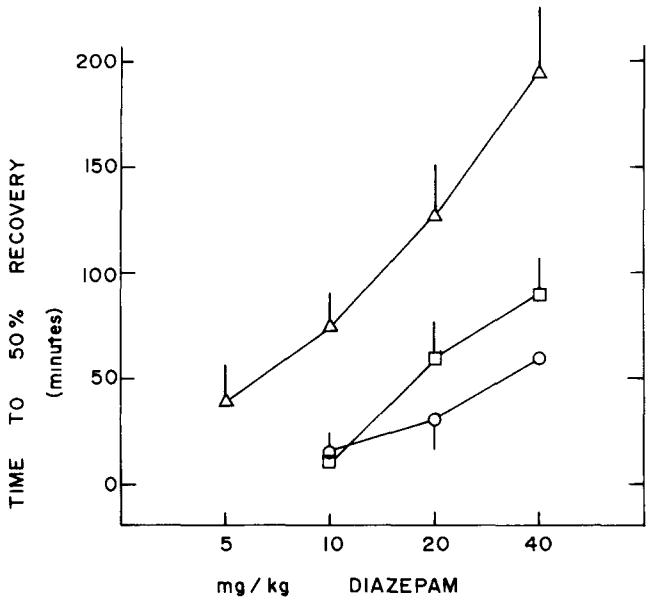

FIG 4 The dose-response effect of DZ on rotarod performance in control chronic PB-treated and chronic MQ-treated rats The times (min) to 50% recovery of rotarod performance produced by various doses of DZ are plotted for control (△), chronic PB-treated (○) and chronic MQ-treated (□) subjects Both chronic treatments significantly attenuated the effects of DZ as indicated by the shift to the right in the dose-response curves See Fig 2 legend for other details

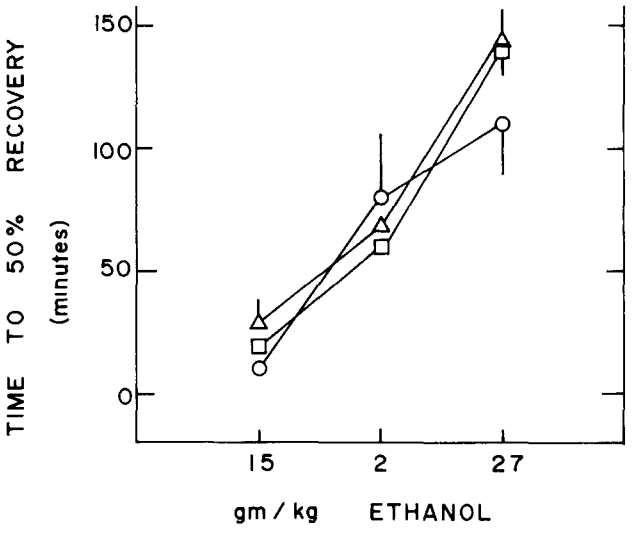

FIG 5 The dose-response effect of ET on rotarod performance in control chronic PB-treated and chronic MQ-treated rats The times (min) to 50% recovery of rotarod performance produced by various doses of ET are plotted for control (△) chronic PB-treated (○) and chronic MQ-treated (□) subjects Each point and vertical bar represent the mean±S E M from at least six subjects Neither chronic treatment significantly altered the dose-response curve for the effect of ET See Fig 2 legend for other details

chronic PB rats as compared to the chronic MQ animals, but this did not reach significance

The ET test doses (Fig 5) tended to have a decreased duration of effect in chronic PB rats and an increased duration in the chronic MQ group, especially at the highest dose of ET However, none of the doses of ET showed a significant difference in the pattern of rotarod disruption after the chronic drug treatments, relative to the effects in the control group

The levels of the injected doses of each test drug were chosen to produce comparable intensities of drug depression Unfortunately, at these dose levels the agents differed prominently with regard to the duration of RR impairment, complicating the analysis of tolerance development To offset this disadvantage, we determined the relative tolerance levels achieved by calculating the ratio of the duration of RR impairment in control subjects to that in chronic PB-or chronic MQ-treated rats for each test dose injected (Table 2) The values obtained again indicate that PB and MQ exhibit prominent tolerance and cross-tolerance with either chronic treatment Cross-tolerance to DZ is less dramatic after either chronic treatment However, ET failed to show a clear pattern of cross-tolerance after chronic PB or chronic MQ treatment From the data of Table 2 it is obvious that the largest dose of ET tested, 2 7 g/kg, showed no evidence for cross-tolerance as a function of chronic PB, or chronic MQ treatments Nevertheless, the duration of effect of this test dose of ET was similar to those of the lowest test doses of PB and MQ in control rats (approximately 150 min), although these latter doses administered to rats receiving chronic drug treatments exhibited a marked cross-tolerance (10- to 20-fold decrease in duration of effect)

TABLE 2

RELATIVE TOLERANCE LEVELS TO RR IMPAIRMENT BY PB MQ DZ AND ET IN CHRONIC PB- AND CHRONIC MQ-TREATED RATS*

Test Drug and Dose		Ratio of Effect† Control/ Chronic PB	Control/ Chronic MQ
PB	10 mg/kg	22 0	19 9
	20 mg/kg	9 4	5 5
	40 mg/kg	5 2	5 7
MQ	14 3 mg/kg	10 3	16 0
	24 mg/kg	2 7	3 4
	40 mg/kg	2 0	2 6
DZ	10 mg/kg	4 5	5 9
	20 mg/kg	4 0	2 1
	40 mg/kg	3 3	2 2
ET	1 5 g/kg	2 6	1 6
	2 0 g/kg	0 9	1 2
	2 7 g/kg	1 3	1 1

*RR=rotarod performance, PB=pentobarbital, MQ=methaqualone DZ=diazepam, ET=ethanol

†Ratio of min to 50% recovery of RR performance in control rats over min to 50% recovery in chronic PB- or MQ-treated subjects for each test drug dose examined

DISCUSSION

The degree of tolerance and cross-tolerance after these chronic treatments was dramatic for PB and MQ and significant for DZ However, no cross-tolerance was noted to ET

These results that illustrate a lack of cross-tolerance to ET are in contrast to a number of reports in which cross-tolerance has been observed [9,11] This discrepancy probably relates to the duration of chronic treatments, since longer chronic exposures would be more likely to facilitate the development of functional tolerance and cross-tolerance than these brief exposures [6,9] Alternatively, the lack of ET cross-tolerance may relate to the particular behavior measured in the present study

Chronic barbiturate or MQ treatments have been shown to increase liver microsomal metabolic capacity with short-term treatments [6, 9, 12], and PB, MQ and DZ appear to be metabolized largely by hepatic microsomal enzyme activity [4] The role of hepatic microsomal metabolism in the disposition of ET is probably much less important [14] Therefore, the tolerance and cross-tolerance to PB, MQ and DZ observed in the present study may be largely pharmacokinetic in character Indeed, Commissaris et al [3] have demonstrated that dramatic tolerance to the hypothermic effect of PB produced by the same chronic PB regimen as used in the present study is predominantely dispositional and not cellular in nature In this previous study the one week of chronic PB did not affect the brain PB concentrations associated with varying intensities of the hypothermic response after various PB test doses, although the duration of the effect for each test dose was greatly shortened along with a decreased serum half-life It seems likely that these data can be related to the present results, at least for PB, since Ho [5] indicated that the general CNS depressant effects (sedation, ataxia) and hypothermic response due to PB, and tolerance thereto, were well correlated In addition, none of the chronically-treated subjects in this study exhibited signs of withdrawal after stopping the drug treatment, and a specific type of withdrawal phenomenon is associated with the discontinuation of chronic drug administration of a general CNS depressant in functionally tolerant subjects [6, 7, 9]

Although the comparison of tolerance and cross-tolerance data for the 4 drugs was complicated by the different durations of action of dose levels showing a comparable intensity of action, comparison of the ratios of times to 50% recovery of RR performance in control vs chronic drug-treated subjects yields a measure of relative tolerance levels (Table 2) This analysis supports the contention that cross-tolerance between PB and MQ is marked, particularly at lower dose levels that would emphasize pharmacokinetic changes It was also apparent that cross-tolerance to DZ by this criterion was not as prominent, and that ET ratios indicated no cross-tolerance for this agent

In an earlier study of PB tolerance development to RR impairment [2], even a 3-week chronic treatment with PB in the diet (from 1 to 4 mg/g of powdered chow but without chronic PB injections as in the present study) failed to show much more tolerance than demonstrated here In addition, ET in this earlier study was shown to impair RR in the chronic PB subjects to a very slightly less extent than that in the control animals Thus, only a slight degree of cellular tolerance appeared to develop even with this longer duration of chronic PB treatment Furthermore, this previous study demonstrated that a minor but significant proportion of the tolerance observed after one week of chronic PB involved a behavioral tolerance component [2]

In conclusion, these results indicate that a short duration of chronic treatment with PB or MQ produces a prominent tolerance and cross-tolerance to RR impairments induced by test doses PB and MQ, and to a lesser extent a cross-tolerance to DZ However, no cross-tolerance to ET was demonstrated in either the chronic PB- or MQ-treated group

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