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Atropine in the Abstinence After Chronic Barbital Treatment in the Rat

GÖRAN WAHLSTRÖM

Department of Pharmacology, University of Umeå, Sweden

WAHLSTRÖM, G. *Atropine in the abstinence after chronic barbital treatment in the rat.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 249–255, 1980.—Since cholinergic mechanisms seem to be involved in the changes induced by chronic barbital treatments in male rats, various treatments with atropine were given during the abstinence after 32–33 weeks of exposure to barbital (200 mg/kg/day). The effects of the atropine treatment were recorded as a tolerance towards a hexobarbital anaesthesia threshold. In Experiment 1, 1.5 mg/kg/day of atropine was given on Days 23–29 after the end of the barbital treatment. Two weeks after the end of the atropine treatment a significant tolerance (+ 16%, Fig. 1A) was seen in barbital-treated animals given the atropine treatment (group BA), but not in the corresponding control groups (group BS, CA and CS). In Experiment 2, the atropine dose was 4 mg/kg/day and the treatment was given on Days 29–44. A tolerance (+ 20%, Fig. 4) with maximum 2 weeks after the end of the atropine treatment was recorded in the animals given the combined treatment (group BA). In both experiments a single dose of atropine given on Day 3 reduced this tolerance. In Experiment 3 the atropine treatment was 4 mg/kg/day on Days 3–12. A tolerance above that induced by the barbital treatment (+ 16%, Fig. 5) was recorded three weeks after the end of the atropine treatment (group BA). In this group there was also recorded a new tolerance much later (Day 80). Since a tolerance was induced by atropine, only in previously barbital-treated animals, a carry-over of some change in cholinergic mechanisms is probably involved.

Atropine Barbital Rats Chronic treatments Barbiturate tolerance
Barbital atropine interaction Hexobarbital threshold

DRUGS which can induce anaesthesia, among them barbiturates, are usually regarded to cause their effect on the central nervous system (CNS) by a direct action on the neuronal membrane [15]. The transmitters in the CNS are also influenced, and thirty years ago it was shown that barbiturates could increase the level of acetylcholine (ACh) [4]. Later studies made it clear that not only the level but also the utilization of ACh was affected (reviews: [11,20]).

Barbiturates belong to the group of drugs which can induce dependence [6], and the acute effects of barbiturates on cholinergic activity could be involved also in the changes induced by chronic exposure. In a series of experiments with barbiturates from this laboratory [23], this idea was substantiated, and ACh was implicated not only in some changes found in acute experiments but also in changes induced by chronic treatments. A remarkable connection between barbiturates and cholinergic mechanisms in the CNS was revealed when it was shown that atropine could increase the sensitivity of normal rats only to the more potent of the two optical isomers of hexobarbital [22]. Recent extended studies of the biochemical changes in the brain during the first part of the abstinence after chronic barbiturate treatment (Days 0–30 after the end of a barbital treatment) revealed an increase both of the regional biosynthesis of ACh [12] and the number of muscarinic receptors [14] in the CNS. Studies later in the abstinence (53 days after the end of a barbital treatment) revealed an increased sensitivity to choline, and still later (81–83 days after the end of a barbital treatment) a decrease in ACh biosynthesis mainly restricted to the cortex preparation [13]. In the striatum the level of ACh seems to be decreased at least up to Days 81–83 [12,13].

With this background it is evident that the effects of atropine when administered during abstinence after chronic barbiturate treatments are of interest. Atropine in a single administration on Day 3 of abstinence will reduce tolerance towards hexobarbital, and also slightly reduce convulsive frequency [21,22]. In the experiments to be presented here, the atropine treatments were extended and covered a large part of the early abstinence, but still were administered during a part of abstinence in which, as discussed above, function in the cholinergic part of the brain has not returned to normal. Some of the results have been presented elsewhere in preliminary form [23,24].

METHOD

General Information

Male Sprague-Dawley rats (Nih/Han/Mol, Möllegaard, Li. Skensved, Denmark) were used in all exeriments. At the start of each experiment the body weights of the animals were around 300 g. They were kept three per cage in an animal room with a temperature around 25°C and with a reversed 12D:12L artificial lighting schedule. Light off was at 08.00 and light on at 20.00. All external lights were excluded in the rat rooms. Food and drinking fluid were available at all times.

During the treatment with sodium barbital a solution of

sodium barbital (3.33 mg/ml) was the only drinking fluid. The doses were calculated as weekly averages from the fluid consumption. The controls were given water instead of the barbital solution. At the end of the treatment the barbital solution was changed to water. This was performed on Day 0 of the abstinence, and days in the abstinence were then numbered consecutively. Details on this kind of treatment are given elsewhere [18]. Atropine was administered IP once a day. This dose was given during the first hours of activity of the rats. Doses between 1 and 8 mg/kg were used. The higher doses can influence a hexobarbital threshold (see below) in acute experiments [19] in normal rats.

The sensitivity of the CNS to barbiturates was tested with a hexobarbital threshold. During the test animals were infused with sodium hexobarbital (15 mg/kg/min, volume rate 0.1 ml/min). The electroencephalogram was recorded during the infusion. The criterion of induced depression in the CNS was the first burst suppression which lasted 1 sec or more. The dose of hexobarbital needed to induce this criterion was the measure of sensitivity of the CNS. All threshold tests were performed during the first hours of darkness. Since rats have individual differences in sensitivity to hexobarbital when tested in this manner [16] all threshold doses are given as percent of an average individual pre-experimental threshold determined prior to any treatment. The pre-experimental threshold was based on two or three determinations of the hexobarbital threshold in each rat.

The general design of all experiments was similar. After determination of the pre-experimental average a barbital treatment was started in half of the participating animals. At the end of this treatment the animals were used in other experiments during the time of maximal withdrawal which occurs around Day 3 [18]. These experiments consisted of hexobarbital threshold determinations and sometimes acute atropine administrations. These administrations were incorporated in the design of the present experiments which as a main feature had a long atropine treatment. This treatment was given IP to approximately 50% both of the animals earlier treated with barbital and of the controls. Saline was given to the rest of the animals. All animals were randomly allocated to the different treatments. At the end of the long atropine treatment several hexobarbital thresholds were performed, usually at weekly intervals. All experiments reported here contain the following four main groups: Group BA, barbital treated animals also given the long atropine treatment. Group BS, barbital treated animals given saline instead of atropine. Group CA, control animals only given the atropine treatment. Group CS, control animals given saline instead of atropine.

If not stated otherwise two-tailed probabilities determined with student's *t*-test are given in statistical comparisons of means. Since increased thresholds were expected probabilities less than 0.1 have been included in Table 1 and 2. Probabilities above 0.1 have been regarded as nonsignificant. N indicates number of animals.

All doses are given as the corresponding salts.

Experiment 1

The barbital treatment in this experiment lasted 33 weeks. During the last 5 weeks of the treatment the animals drank approximately 190 mg/kg/day of barbital. The atropine treatment was given from Day 23 to Day 29. The dose was 1.5 mg/kg/day. The design includes the four main groups and two additional groups. Group BAA consisted of animals

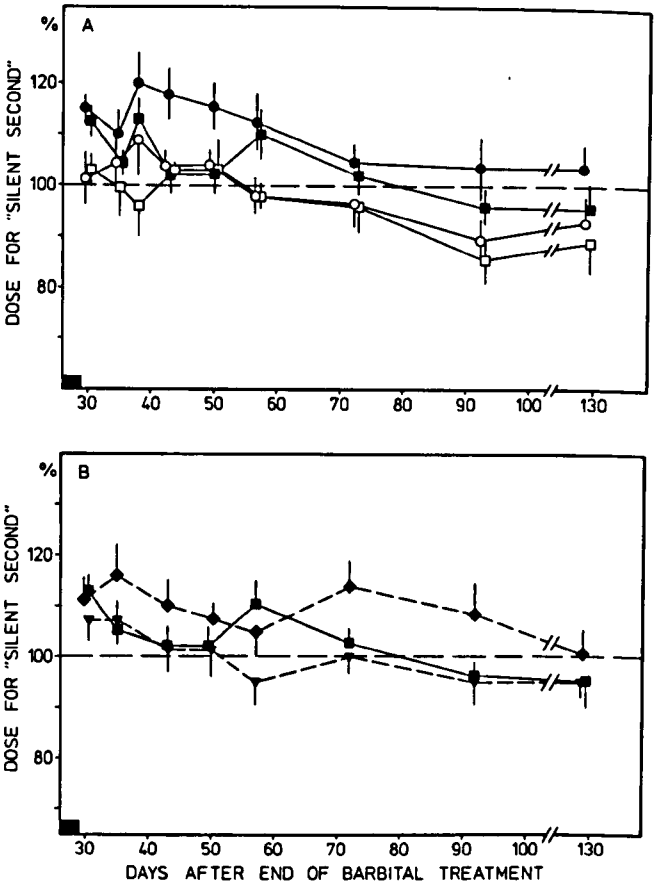

FIG. 1. Results of the hexobarbital threshold determination in Experiment 1. The dose of hexobarbital is given as percent of a pre-experimental value (see METHOD). Previously barbital-treated animals (33 weeks) are indicated by filled symbols. This treatment was given to group BS, BA, BAS and BAA (see METHOD). The end of the atropine treatment (1.5 mg/kg/day for 7 days) is indicated by the filled bar on the abscissa. This treatment was given to group BA, CA and BAA. Groups BAS and BAA were given a single atropine dose (1.0 mg/kg) on Day 3 in the abstinence. In panel A results from group BS (filled square), BA (filled circle), CS (unfilled square) and CA (unfilled circle) are given. In panel B results from group BS (filled square), BAS (filled triangle) and BAA (filled diamond) are given. The standard error (sometimes plotted in only one direction) is given together with each point. The statistical evaluation of the differences between the groups is shown in Table 1. More detailed information on the groups and treatments is given in METHOD.

which after the barbital treatment had been given a single dose of atropine (1.0 mg/kg) on Day 3 and then treated with atropine as described above. Group BAS consisted of animals which after the barbital treatment were treated with atropine (1.0 mg/kg) on Day 3 and then were given saline during the long atropine treatment. Hexobarbital thresholds were determined during the abstinence prior to the long atropine treatment and also on two occasions during the atropine treatment. The results of these tests will not be reported here.

The pre-experimental values of the hexobarbital thresholds were, in group BA 56.3 ± 1.7 mg/kg (N=13); in group BS 60.2 ± 1.5 mg/kg (N=11); in group CA 58.3 ± 2.7 mg/kg (N=9); in group CS 56.6 ± 2.5 mg/kg (N=8); in group

TABLE 1

TWO-TAILED PROBABILITIES OF SIGNIFICANT DIFFERENCES BETWEEN HEXOBARBITAL THRESHOLDS IN THE GROUPS PARTICIPATING IN EXPERIMENT 1

Means* Compared	Day in the Abstinence							
	30	38	43	50	57	73	93	129
CA vs CS	NS	NS	NS	NS	NS	NS	NS	NS
BA vs BS	NS	NS	<0.02	<0.05	NS	NS	NS	NS
BA vs CA	<0.02	NS	<0.10	<0.10	<0.10	NS	<0.05	NS
BA vs CS	<0.01	<0.025	<0.05	NS	<0.10	NS	<0.05	<0.10
BS vs CS	<0.05	<0.05	NS	NS	<0.10	NS	<0.10	NS
BS vs BAS	NS	—	NS	NS	<0.05	NS	NS	NS
BS vs BAA	NS	—	NS	NS	NS	<0.10	<0.10	NS

NS=$p>0.10$.
—=No data available in group BAS and BAA.
*Means are given in Fig. 1.

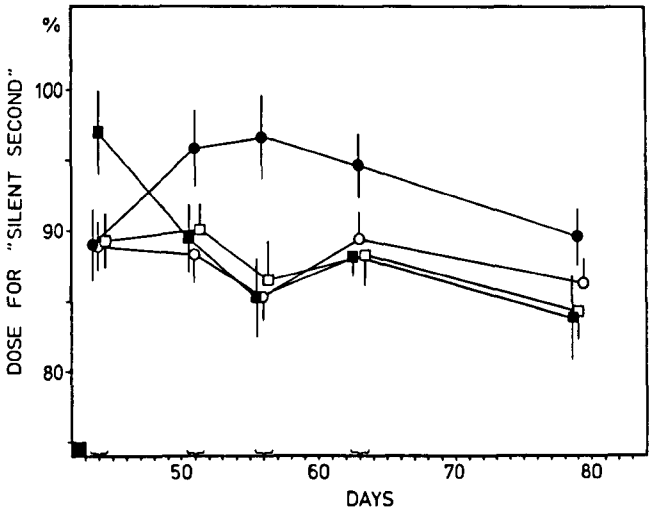

FIG. 2. Result of the hexobarbital threshold determinations in all animals used in Experiment 2. The dose of hexobarbital is given in percent of a pre-experimental value (see METHOD). Previously barbital-treated animals (33 weeks) are indicated by filled symbols. This treatment was given to group BS and BA. The end of the atropine treatment (4 mg/kg/day for 15 days) is indicated by the filled bar on the abscissa. This treatment was given to group BA and CA. The different groups are indicated in the following manner: group BS (filled square), BA (filled circle), CS (unfilled square) and CA (unfilled circle). The standard error (sometimes plotted only in one direction) is given together with each point. More detailed information on the groups and treatments is given in METHOD.

BAA 58.2 ± 2.0 mg/kg (N=14); and in group BAS 59.6 ± 1.3 mg/kg (N=10).

Experiment 2

The barbital treatment in this experiment lasted 33 weeks. During the last 5 weeks of the treatment the animals drank approximately 200 mg/kg/day of barbital. The atropine treatment was given from Day 29 to Day 43 of the abstinence. The dose was 4.0 mg/kg/day. The design included measuring the effects of 0, 2, 4 and 8 mg/kg of atropine on the hexobarbital threshold on Day 3. These doses were given both to controls and barbital-treated animals after random allocation. Further testing of hexobarbital thresholds without pre-treatment was performed prior to the start of the long atropine treatment. On Day 28 a new hexobarbital threshold was determined after the same atropine treatment as on Day 3. In the animals going to participate in the long atropine treatment additional doses of atropine were given later on Day 28 to give a total daily dose of at least 4 mg/kg. (The animals tested with 8 mg/kg started the atropine treatment with this higher dose.) The results of these hexobarbital thresholds have been reported earlier [21]. On Day 35 a last test with hexobarbital after pre-treatment with different doses of atropine was performed. Also on this day additional doses of atropine were given to the animals participating in the long atropine treatment to reach a daily dose of at least 4 mg/kg.

The pre-experimental values on the hexobarbital thresholds were: in group BA, 67.5 ± 1.3 mg/kg (N=22); in group BS, 66.9 ± 1.1 mg/kg (N=19); in group CA, 63.5 ± 0.9 mg/kg (N=30); and in group CS, 66.4 ± 1.0 mg/kg (N=29).

Experiment 3

The barbital treatment in this experiment lasted 32 weeks. During the last 5 weeks of the treatment the animals drank approximately 200 mg/kg/day of barbital. The atropine treatment started with 8 mg/kg on Day 3. This dose was given in connection with a hexobarbital threshold, and the results have been reported earlier [22]. The atropine dose after that was 4 mg/kg/day and the last dose was given on Day 12. This experiment only contained four main groups.

The pre-experimental values on the hexobarbital thresholds were: in group BA, 68.0 ± 3.4 mg/kg (N=9); in group BS, 71.4 ± 1.6 mg/kg (N=9); in group CA, 68.3 ± 1.6 mg/kg (N=12); and in group CS, 68.8 ± 1.4 mg/kg (N=12).

RESULTS

The results of the hexobarbital thresholds determined in the main groups of Experiment 1 after the end of the atropine treatment (7 days with 1.5 mg/kg/day) are shown in Fig. 1A. The corresponding p values on the differences between the participating groups are given in Table 1. The atropine treatment had no significant effect on the control groups

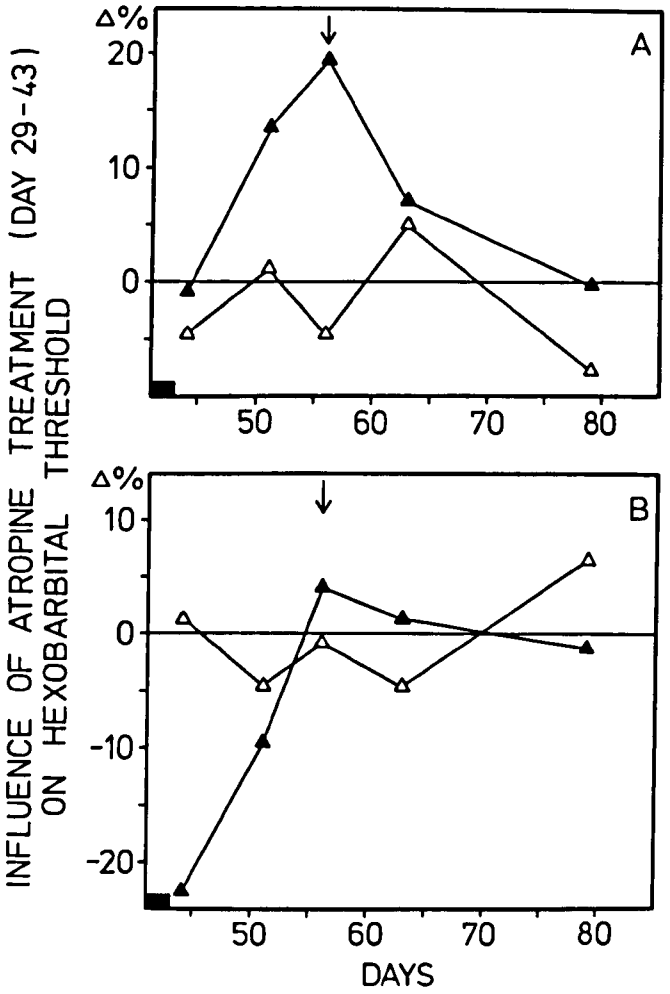

FIG. 3. Results of hexobarbital threshold determinations in the subpopulations of animals in Experiment 2 which were given saline (A; upper panel) or 8 mg/kg of atropine (B; lower panel) on Day 3 in the abstinence. The hexobarbital thresholds are given as differences (delta percent) between animals given the atropine treatment (4 mg/kg/day for 15 days between Day 29 and Day 43) and those given saline during the same time. Differences in barbital-treated animals (group BA—group BS) are indicated by filled symbols, differences in controls (group CA—group CS) by unfilled symbols. The end of the atropine treatment is indicated by the bar on the abscissa. The minimum number of animals on which the differences were calculated was 10 barbital-treated and 10 control animals in panel A and 11 barbital-treated and 12 control animals in panel B. The arrows indicate the values used in Fig. 4.

which had not had the barbital treatment (CS vs CA, Table 1). Group BS, which had only had the barbital treatment, showed on Day 30 a tolerance which probably is part of a continuum present since the end of the barbital treatment. After periods with thresholds in the control region, with the first one seen on Day 35, tendencies towards a new appearance of tolerance were again recorded on Day 38, Day 57 and Day 93 (BS vs CS). Up to Day 43 group BA, which in addition had the 7 days of atropine treatment, behaved approximately as group BS (BA vs BS, Table 1). On day 43 and 50 group BA was distinctly different from group BS, and on Day 43 group BA also differed from the controls (BA vs CA and BA vs CS, Table 1). This tolerance in relation to the controls was still present on Day 57, at a time when a tolerance could have reoccurred in group BS (BS vs CS). On Day 73 no significant tolerance was seen, but on Day 93 some changes were again seen in group BA. Thus, 7 days of treatment with atropine had no effect in control animals, but gave a tolerance above that which was induced by the long-term barbital treatment as such. This tolerance, probably induced by atropine in animals made sensitive by the barbital treatment, was most marked 2–3 weeks after the end of the atropine treatment. On Day 93, which is more than 60 days after the end of the atropine treatment, a tendency towards renewal of tolerance could be traced in animals given both the atropine and barbital treatments.

The possible long-term effects of a single dose of atropine (1 mg/kg) on Day 3 is illustrated in Fig. 1B. The relevant statistical comparisons are given in Table 1. This dose of atropine had no acute effect on the hexobarbital thresholds determined on Day 3 (data not shown). Higher doses of atropine will reduce tolerance on Day 3 [21]. In the present experiment group BAS, which was a barbital-treated group given only the single atropine dose on Day 3, had hexobarbital thresholds similar to group BS on all test occasions except on Day 57. The tendency towards a renewed tolerance seen in group BS was not seen in group BAS (BS vs BAS, Table 1). In group BAA, which had been given both the single atropine dose on Day 3 and the atropine treatment on Days 23–29, no significant tolerance was seen on Day 43 and 50 at a time when such a tolerance was seen in group BA. The opposite tendency was seen on Day 73 and 93. Thus the most consistent late effect of a single dose of atropine given at the time of maximal withdrawal seemed to be a reduction of tolerance both in animals only treated with barbital (Day 57) and in animals given the combined treatment with barbital and atropine (Day 43 and 50).

The overall result of Experiment 2 with no consideration taken of the possible influence of the single dose of atropine given on Day 3, is shown in Fig. 2. The atropine treatment given to group BA and CA lasted in this experiment from Day 28 to Day 43 (16 days) and the dose was 4 mg/kg/day. It is clear that this more extensive treatment with atropine had no effect on the controls (group CS vs group CA). In group BS a significant tolerance still remained on Day 44 (group BS vs CS, $p<0.05$). No further difference from the controls was seen in this group. In group BA, given both the barbital and the atropine treatments, there was no tolerance on Day 44. In fact group BA was on this day significantly ($p<0.05$) different from group BS. On Day 56, which was 13 days after the end of the atropine treatment, a new maximum in tolerance had developed in group BA (BA vs CS, $p<0.02$, BA vs CA, $p<0.001$). This tolerance was not seen in group BS (BA vs BS, $p<0.01$). On Day 79 the tolerance was again reduced and no significant differences were found when group BA was compared with group BS and CA. However, it is not possible to state that the thresholds in group BA were back to the control level, since a marginal significance ($p<0.1$) still remained when group BA was compared with group CS.

The influence of the single atropine dose given on Day 3 in Experiment 2 has been evaluated in Figs. 3 and 4. These results are given as differences between the animals which had the atropine treatment on Day 28–43 and the corresponding saline-treated animals. Due to the testing design, the saline was substituted with atropine on Day 28 and 35 (see *Method*). Four subpopulations can be obtained depending on the single doses of atropine. Figure 3A shows the results in

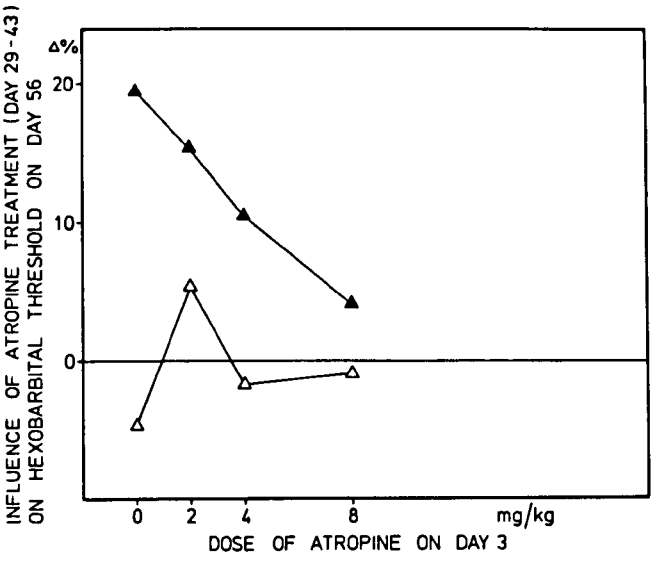

FIG. 4. Relationship between the dose of atropine given on Day 3 and the maximal tolerance obtained in Experiment 2 after the end of the longer atropine treatment (4 mg/kg/day between Day 29 and 43) in barbital-treated animals (filled symbols) and controls (unfilled symbols). Day 56 was considered as the day with maximal tolerance (indicated by arrows in Fig. 3). The ordinate represents a difference in hexobarbital threshold between atropine- and saline-treated animals. The values obtained in the subpopulations of animals given 0 and 8 mg/kg on Day 3 are shown in Fig. 3 (A and B, respectively).

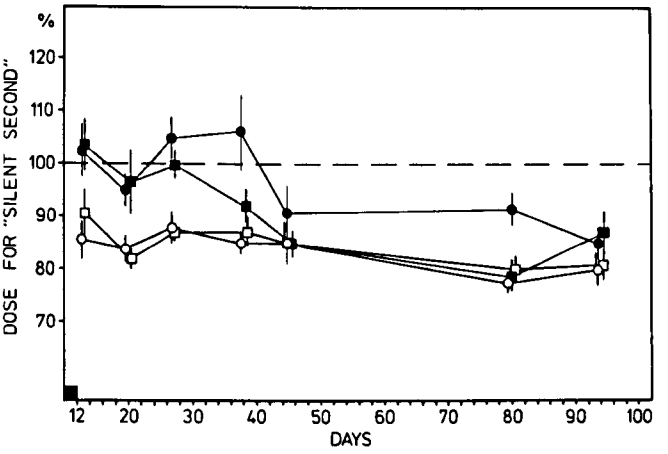

FIG. 5. Results of the hexobarbital threshold determinations in Experiment 3. The dose of hexobarbital is given in percent of a pre-experimental value (see METHOD). Previously barbital-treated animals (33 weeks) are indicated by filled symbols. This treatment was given to group BS and BA. The end of the atropine treatment (4 mg/kg/day for 10 days) is indicated by the filled bar on the abscissa. This treatment was given to group BA and CA. The different groups are indicated in the following manner: group BS (filled square), BA (filled circle), CS (unfilled square) and CA (unfilled circle). The standard error (sometimes plotted only in one direction) is given together with each point. The statistical evaluation of the differences between the groups is shown in Table 2. More detailed information on the groups and treatments is given in METHOD.

the subpopulation of animals which were not given any atropine on Day 3. The curve for these barbital-treated animals is more distinct with a more marked maximum on Day 56 when compared to the curve for all animals (group BA, Fig. 2). In Fig. 3B is shown the corresponding subpopulation which had been given 8 mg/kg of atropine on Day 3. No difference was now recorded on Day 56 in the previously barbital-treated animals compared to controls. This was not due to an increase in thresholds in the saline-treated animals which had also received atropine on Day 28 and 35. Thus the tolerance in the atropine-treated animals was reduced by the single dose of atropine given on Day 3. This result is similar to the reduction of tolerance obtained with a smaller atropine dose in group BAA in Experiment 1. A negative difference in the barbital-treated animals was found on Day 44 (Fig. 3B). This indicated that barbital-treated animals given 8 mg/kg of atropine on Day 3 and then the atropine treatment on Day 28–43 had low thresholds on Day 44 if compared with animals given barbital and single doses of atropine on Day 3, 28 and 35. A comparison of the thresholds for barbital-treated animals on Day 44 which are shown in Figs. 3A and B, indicates that the loss of tolerance seen in group BA on Day 44 in Fig. 2 is due to the differences in the subpopulations treated with various doses of atropine on Day 3.

Figure 4 shows the differences obtained on Day 56 in all subpopulations with doses of atropine given on Day 3 as independent variable. Day 56 was chosen as the day when tolerance which could be induced by an atropine treatment on Days 28–43 had a maximum (Fig. 2 and 3A). This tolerance was clearly influenced in a dose-dependent manner by a single dose of atropine given on Day 3, with a significant dose response curve ($r=0.995$, $b=-1.93$, $DF=2$, $p<0.01$). Eight mg/kg of atropine as a single dose on Day 3 can almost completely eliminate the tolerance which could be induced by the late atropine treatment.

The results obtained in Experiment 3 are shown in Fig. 5. The corresponding p values on the differences between the participating groups are given in Table 2. Again, in this experiment, the atropine treatment with a duration of 10 days (4 mg/kg/day) did not influence the thresholds in the controls (CA vs CS). Since atropine treatment was started on Day 3 of abstinence, more of the tolerance induced by the barbital treatment was displayed by group BS when compared with the corresponding groups in Experiments 1 and 2. Significant differences (BS vs CS, Table 2) were recorded up to Day 27. Group BA showed a similar pattern as BS during this part of the abstinence. In addition a tolerance was also recorded on Day 38 in group BA. In this group there was furthermore a clear renewal of tolerance on Day 80 which was 68 days after the end of the atropine treatment, and thus at approximately the same time after the atropine treatment as the corresponding late change was found in Experiment 1 (Fig. 1A).

DISCUSSION

After exposure to a substance which can induce drug dependence some long-lasting changes in the central nervous system (CNS) can remain after the end of the acute withdrawal. These changes need not give overt symptoms, but it is sometimes possible to reveal their presence by a more rapid induction of physical dependence during a second exposure. This phenomenon has been denoted carry-over [5].

To use the original substance during the second treatment is the most straightforward way to study carry-over. Such a carry-over has been demonstrated several times with ethanol as the inducing substance [1, 2, 3, 7, 25]. Some sign of exci-

TABLE 2

TWO-TAILED PROBABILITIES OF SIGNIFICANT DIFFERENCES BETWEEN HEXOBARBITAL THRESHOLDS IN THE GROUPS PARTICIPATING IN EXPERIMENT III

Means* Compared	Day in the Abstinence						
	13	20	27	38	45	80	94
CA vs CS	NS	NS	NS	NS	NS	NS	NS
BA vs BS	NS	NS	NS	<0.10	NS	<0.02	NS
BA vs CA	<0.02	<0.02	<0.005	<0.01	NS	<0.005	NS
BA vs CS	<0.10	<0.005	<0.001	<0.02	NS	<0.01	NS
BS vs CS	<0.10	<0.05	<0.001	NS	NS	NS	NS

NS=$p>0.10$.
*Means are given in Fig. 5.

tation has usually been the measured variable. Carry-over can also be shown with barbital as the inducing agent and tolerance to hexobarbital as the measured variable [17]. In one experiment in this series ethanol was used instead of barbital as the second substance to which the animals were exposed. A carry-over between barbital and ethanol could be established [17]. This last experiment indicates that there is a possibility to use carry-over as a tool in more elaborate pharmacological explorations of these late changes in the intact animal. The results of the present study can be regarded as such an expansion of the use of carry-over in the study of drug dependence. The principle in the test procedure used has similarities with the manner in which cross-tolerance is tested, but it is probably at present less confusing if a clear distinction is kept between carry-over to other substances and cross-tolerance. The main criterion of distinction between the two phenomena is, however, only the time in the abstinence when treatment and testing are done. This is not a very satisfactory demarkation.

In all three of the present experiments atropine could induce a tolerance to hexobarbital only in animals pretreated with barbital for 33 weeks. This means that the carry-over probably is due to a change in a cholinergic mechanism which is still present at the time of atropine treatment and gives the animal a specific sensitivity to this treatment. Furthermore, the fact that the effect of the treatment could be measured as a change in a hexobarbital threshold strengthens the tie between barbiturates and cholinergic mechanisms discussed in the introduction. Whether the barbital treatment is mandatory or if more intense treatment with atropine can also produce the same tolerance to hexobarbital in normal animals is at present unknown. This question could have clinical significance, since there are sporadic case reports on abuse of atropine in the human [8, 9, 10], and there could be a relation between abuse of atropine and prior exposure to other drugs of dependence.

Experiments 1–3 are not a systematic study of all variables which could influence the result. Both the intensity and duration of the barbital treatment is probably involved. Two other apparent variables are the dosage and duration of the atropine treatment. Since time after the barbital treatment also could be important, the duration of the atropine treatment will be hard to evaluate properly. As can be seen from Figs. 1 and 5, the presence of tolerance in group BS also could confound the possibility of evaluating the effects of atropine in more detail. Nevertheless the present experiments allow some evaluation of the situation. The barbital treatments were similar in Experiments 1–3. Experiment 2, with supportive evidence from Experiments 1 and 3, shows that the maximal effect is seen two to three weeks after the end of the atropine treatment. The reason for this delay is not known. The maximal tolerance in animals given the barbital and the atropine treatments was approximately 20% above the control level (Figs. 1A, 3A and 5). The control level below 100% seen in Experiments 2 and 3 and the control level seen in Experiment 1 are probably due to the age of the animals. The result in Experiment 1 with the shortest atropine treatment (7 days) and with the lowest dose (1.5 mg/kg/day) was not distinctly less than the results in Experiments 2 and 3. This could mean that the differences in durations and doses used here were not important or that time after the end of the barbital treatment is a more critical variable with the highest sensitivity around Days 20–30 after 33 weeks of barbital treatment.

A study of the regional biosynthesis of choline [12] revealed no change from controls in barbital-treated animals on Day 12 and Day 30 in the abstinence [12]. Three parts of the brain were studied (striatum; cortex and hippocampus; midbrain, medulla oblongata, and cerebellum). However, a decrease in endogenous ACh in the striatum was seen 12 and 30 days after the end of the barbital treatment. Later in the abstinence (Days 81–83) a change in biosynthesis of acetylcholine has also been reported [13]. Thus there are indications that all parts of the cholinergic mechanisms in the brain are not normal during the time periods when the atropine treatments have been given, but at present no distinct change which is responsible for the present results can be pointed out.

Due to the need to get optimal information out of these chronic experiments a single atropine administration was given as part of other experiments at the time of maximal abstinence on Day 3. The acute effects of this single dose have been reported elsewhere, but both Experiment 1 (Fig. 1) and 2 (Fig. 4) showed that such a single dose of atropine could reduce the maximal tolerance to hexobarbital induced by a later atropine treatment in the barbital-treated animals. In these two experiments the time interval between the single administration on Day 3 and the start of the long-term atropine treatment was 20–26 days. Results from another experiment [13] have indicated that a similar reduction of late

effects of a barbital treatment can be found after such a single dose of atropine when choline thresholds, brain weights and choline utilization are recorded 53–84 days after the end of the barbital treatment. In this connection the results obtained by the treatment with atropine in Experiment 3 are also of interest, since this treatment started with the first dose on Day 3. An increased tolerance of the same magnitude as that induced by the atropine treatment in Experiments 1 and 2 was recorded three weeks later. The effects of a single dose on Day 3 could be different from a dose included in a longer treatment. Further studies are needed to elucidate this point. Whether the marked tolerance on Day 80 in Experiment 3 (Fig. 5) is in any way dependent on a treatment on Day 3, thus supporting the results in group BAA in Experiment 1 (Fig. 1B), is another open question.

Several questions are thus unanswered, but it can nevertheless be concluded that the present experiments have established the presence of changes carried over from a chronic barbital treatment and later revealed by an atropine treatment. The barbital treatment has probably induced long-standing changes in the cholinergic part of the central nervous system.

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