AMITRIPTYLINE- AND MIANSERIN-INDUCED CHANGES IN ACQUISITION OF PAIRED-ASSOCIATION LEARNING-TASK

R. LILJEQUIST, T. SEPPÄLÄ & M.J. MATTILA

Departments of Pharmacology and Psychology, University of Helsinki, Helsinki, Finland

1 The double-blind study on twenty healthy students was an attempt at assessing the effects of 2-week's treatment with amitriptyline (25 mg three times a day) and mianserin (10 mg three times a day), each alone or separately inhibited with alcohol (0.5 g/kg) on the immediate memory and on the acquisition of a paired-association learning-task.

2 Amitriptyline impaired both the short-term memory-span and acquisition, and alcohol potentiated these effects. The action of mianserin did not deviate significantly from that of the placebo, and it also failed to interact with alcohol.

3 It is concluded that the decrement in learning capacity, that occurs after the 2-week's treatment with therapeutic doses of amitriptyline, reflects changes in both the intrinsic and the regulatory mechanisms of learning.

Introduction

Previous results from this laboratory (Liljequist, Linnoila & Mattila, 1974) indicate that low therapeutic doses of nortriptyline, given three times a day to healthy human subjects, impaired learning, probably by interfering with the acquisition processes. In these conditions another tricyclic antidepressant chlorimipramine, proved ineffective and even antagonized the alcohol-induced impairment. Of these antidepressants, chlorimipramine affected more peripheral sympathetic nerve terminals, as demonstrated by the tyramine pressor test (Seppälä, Linnoila, Elonen & Mattila, 1975). This effect is probably due to the chlordesimipramine formed from the parent drug during prolonged treatment (Nagy, 1974).

In addition to their affecting the availability of cyclic monoamines on the synapses, antidepressants also have an antimuscarinic effect. This might interfere with cognitive processes, as demonstrated with atropine and scopolamine (Whitehouse, 1964; Buresova, Bures, Bochaneky & Weiss, 1964; Bochaneky & Jarvik, 1967). For this reason we have compared the effects of amitriptyline and mianserin on those variables which represent acquisition stages in learning processes. Mianserin is a tetracyclic compound with clinical antidepressant action (Murphy, 1975; Kafoe & Leonard, 1973). Unlike amitriptyline, it does not block the amine uptake *in vivo* experiments, and it also lacks anticholinergic effects (Raiteri, Angelini & Bertollini,

1976; Ghose, Coppen & Turner, 1976; Coppen, Gupta, Montgomery, Ghose, Bailey, Burns & De Ridder, 1976).

Methods

Subjects and treatments

Twenty healthy subjects, fourteen males and six females, aged 21.1 ± 1.4 years (mean $\pm$ s.d.) and weighing 67.9 ± 9.9 kg, served as paid volunteers. None of the subjects were regularly on drugs nor had a history of psychiatric illness. The participants were informed of the objects and the procedure of the experiment and consented to volunteer the experiment.

Mianserin hydrochloride (10 mg) (Tolvon®, Bolvidor®, Organon), amitriptyline hydrochloride (25 mg) (Tryptizol®, Merck, Sharp & Dohme), or lactose placebo were administered in identical tablets three times a day, double-blind cross-over for 2 weeks each. A one week's drug-free period was allowed between the treatments. The sequence of treatments was ordered according to the Latin square design.

When the subjects came to the testing sessions, they ingested an alcoholic or nonalcoholic bitter drink, the dose of ethyl alcohol being 0.5 g/kg body weight. The drinks were ingested chilled, the drinking time was 10 min. The test began 30 min after the ingestion of drugs.

Figure 1 Effects of amitriptyline (AT), mianserin (M), alcohol (A) and placebo (P) on immediate memory. Means $\pm$ s.d. of the number of digits correctly recalled in the digit memory span test. Data are based on the analyses of nineteen subjects. Statistical differences were calculated by using Student's paired *t*-test (** $P < 0.01$).

Testing session

Before the beginning of the actual tests the subjects were trained in order to perform in this kind of learning situation, and to develop individual learning strategies. The test session began at 16 h 30 min at which time the last capsule of each treatment period and placebo or alcohol beverage were administered.

Learning tests

The immediate memory was assessed by means of a digit series. Ten digits were verbally presented. The subjects were asked to repeat them immediately in the same order after the instructor had finished. The total number of digits correctly recalled and repeated in the correct order is called parameter I.

The measurement of acquisition was assessed by means of a paired association learning task (Hilgard, 1951). Six paired-associate syllables and six stimulus syllables were presented by using a projector. All the CVC syllables were of 0% association value (Hilgard, 1951). Parameter II₁ means the total number of trials necessary to learn the correct associations. Parameter II₂ means the total number of mistakes, or failures to respond.

Assessment of the sedative effects of the drugs

Critical flicker fusion frequency was measured after 1- and 2-week's treatment with the drugs (Bishop, Guthrie & Hornsby, 1969). In this test the subjects'

Figure 2 Effects of amitriptyline (AT), mianserin (M), alcohol (A) and placebo (P) on paired associate learning. Means $\pm$ s.d. of the number of presentation times of the paired association learning task refers to memory acquisition. Data are based on the analysis of nineteen subjects. Statistical differences were calculated by using Student's paired *t*-test (** $P < 0.01$).

ability to discriminate the fusion of a flickering red light (diameter 3 mm) was tested as critical flicker frequency (CFF) at a distance of 110 cm. The subjects wore artificial pupil constrictors in order to eliminate the peripheral effects of drugs on pupil size.

Subjective evaluation

At the end of every drug period the subjects estimated the nature of the treatment as tranquilizing, stimulating, or non-effective by a questionnaire. Moreover, the subjects were asked to report the noticed side-effects like drowsiness and dryness of the mouth.

Pharmacokinetics

Venous blood samples were drawn after each test session for assessment of amitriptyline, nortriptyline, and the sum of their combined plasma levels. The drug plasma levels were assayed by gas liquid chromatography (Seppälä, Liljequist, Linnoila, Fredriksson-Overø & Dorrity, 1977). The detailed description of the method and results have been presented previously (Seppälä *et al.*, 1977).

Statistics

The performance after drugs was compared to the placebo results with the Student's paired *t*-test. The relationship between learning capacity and drug plasma levels were calculated using regression analysis (Hays, 1963).

Results

Significant drug effects on learning were seen, the results are presented in Figures 1, 2, and 3.

Figure 3 Effects of amitriptyline (AT), mianserin (M), alcohol (A) and placebo (P) on paired associate learning. Means $\pm$ s.d. of the number of mistakes during acquisition of paired-associate learning task refers to learning capacity. Data are based on the analyses of nineteen subjects. Statistical differences were calculated by using Student's paired *t*-test (* $P < 0.05$; ** $P < 0.01$).

Immediate memory

When the total number of digits correctly recalled and repeated was measured (parameter I), significant impairment in short-term memory-span was found after the treatment with alcohol ($P < 0.01$), amitriptyline + alcohol ($P < 0.01$) and mianserin + alcohol ($P < 0.01$), compared to placebo effects.

Amitriptyline failed to modify parameter I significantly. Treatment with mianserin resulted in a slight improvement of this performance.

Acquisition of a paired-association learning-task

The 2-week treatment with amitriptyline or mianserin did not modify the total number of trials necessary to learn correct associations (parameter II₁), when compared to placebo results. Neither did acute intake of alcohol alone modify the parameter.

Figure 4 The change in flicker fusion frequency after 1 (□) and 2 (▨) week's treatment with amitriptyline (AT), mianserin (M), and alcohol (A), compared to the placebo values (mean frequency on placebo 10⁴/429 Hz). Statistical differences were calculated by using Student's paired *t*-test. * $P < 0.05$; ** $P < 0.02$, and *** $P < 0.001$ when compared to the values during placebo treatment.

The amitriptyline + alcohol group significantly ($P < 0.01$) deviated from that of the placebo, and mianserin group ($P < 0.01$).

Treatment with amitriptyline, but not with mianserin, significantly ($P < 0.05$) increased the total number of mistakes, or failures to respond (parameter II₂), as compared to the placebo results.

Acute intake of alcohol after amitriptyline ($P < 0.01$) and mianserin ($P < 0.05$), or alcohol alone ($P < 0.05$), all impaired learning, as compared to the placebo results. Treatment with mianserin significantly ($P < 0.01$) deviated from the combined effects of amitriptyline and alcohol.

The plasma levels of amitriptyline, or its metabolite, nortriptyline, or their combined levels did not correlate to the memory impairment after amitriptyline.

Critical flicker fusion frequency

Critical flicker fusion frequency was decreased after each treatment, as compared to the placebo results. Significant decrement was evident after 2-week's treatment with amitriptyline, and when alcohol was administered together with mianserin ($P < 0.02$). One-week's treatment with amitriptyline and mianserin resulted to significant decrease in critical flicker fusion frequency only when these drugs were administered together with alcohol. In these conditions the results differed at $P < 0.001$ significance level from placebo results.

Subjective evaluation

Drowsiness was subjectively reported by fourteen subjects when being on mianserin and by ten subjects when being on amitriptyline and by one subject after placebo. Eight subjects reported dryness of the mouth after amitriptyline and none after mianserin or placebo.

Discussion

The present study demonstrates that amitriptyline in therapeutic doses impairs memory functions, whereas the effects of mianserin do not differ from those of the placebo. The exact nature of the impairment of the cognitive functions remains unanswered until after a more systematic study of memory processes.

During mianserin and placebo treatment, the short-term memory storage was within the normal range, i.e. approximately seven items. After amitriptyline, performance corresponded lower limits to be accepted as normal. Alcohol alone significantly reduced the short-term memory span. The results of the present study, concerning the effects of relatively small, acute doses of alcohol, tally well with previous studies (Liljequist *et al.*, 1974). When alcohol and amitriptyline were administered simultaneously, the short-term memory test indicated momentary impairment in general intelligence (Wechsler, 1944). Since the experimental population consisted of students, we expected their verbal learning capacity to be high or normal. Therefore, amitriptyline and alcohol can impair sequential verbal learning, and if they are acting at the same time, also other cognitive functions are affected.

The performance factors often interfere with the learning capacity. The experimental procedure used in this study excludes the possibility, that the impaired learning capacity during amitriptyline and alcohol treatment resulted from inadequate attention during acquisition, since the subjects had to repeat the response syllable to reach the learning criterion. Nevertheless, the learning scores which measure the acquisition stage (Atkinson & Shiffrey, 1968), were significantly impaired. Since the indication of learning required success in recalling, there is the possibility, that the only stage affected was the retrieval from immediate memory. However, since the other tests (Seppälä, 1977) were adequately performed, this possibility is not probable.

If it is accepted that the formation of the long-term memory traces was impaired, it is still unclear, as to whether this reflects specific memory, or properties of a more general sedative effect of the drugs used. The observation that mianserin in spontaneously reported questionnaires appeared to have more sedative

properties than did amitriptyline, contraindicates the role of general sedative effects. Vigilance, coordination, and reaction capacity appeared unchanged during the learning tests (Seppälä, 1977).

If it is accepted, that amitriptyline and alcohol, at the time of rising blood alcohol concentration, interfere with the process of consolidation, it would be relevant to find the loci of action.

The mechanism of short-term memory has been proposed as reflecting a synaptic transmission efficacy (Eccles, 1964), whereas the formation of memory-trace more specifically depends on the cholinergic mechanisms of the limbic system, and the connections to the cortex and reticular activating system (Il'yuchenok, 1965). Thus, part of the effects of amitriptyline are mediated through inhibition in the cholinergic brain structures, and through a block in the corresponding components of the reticular formation. Consequentially, the cortex is de-afferented, and the spatial and temporal activity between the nerve cells is diminished (Il'yuchenok, 1975). This might manifest as a deterioration of short-term memory span.

Small, single doses of ethanol are (possibly) accompanied by changes in central catecholamine metabolism (Corrodi, Fuxe & Hökfelt, 1966; Carlsson & Lindqvist, 1973). There is evidence that drugs which block catecholamine synthesis (α -methyltyrosine) or storage (reserpine) may impair learning by secondary rather than by primary actions (Izquierdo, 1975). Therefore, the mechanisms of the effects of alcohol on memory are difficult to explain.

The different actions of amitriptyline and mianserin on memory are possible to explain, except by cholinergic properties, also by their action on brain amine metabolism. Mianserin possibly increases the turnover of noradrenaline and, to a lesser degree, of dopamine, thus increasing the availability of noradrenaline to the receptors (Kafae & Leonard, 1973). In contrast, amitriptyline reduces the turnover of both noradrenaline and serotonin by inhibiting the re-uptake of these amines (Kafae & Leonard, 1973). Perhaps due to this property of mianserin, it even fails to induce depression in the stable reticular activating system, as demonstrated by Vargaftig, Coignet, de Vos, Grijzen & Bonta (1971) in rabbits.

Nortriptyline, which is structurally related to amitriptyline, caused significant but weaker impairment than amitriptyline under similar experimental conditions. Chlorimipramine and mianserin behaved in a similar manner, except that a slight alcohol-antagonizing effect was found with chlorimipramine which in a 2-week study showed a strong action to adrenergic nerve function (Liljequist *et al.*, 1974). Therefore, the interpretation done in that study, i.e. the change in noradrenergic tone is an essential property for memory functions, is supported by the results of the present study.

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