THE EFFECTS OF IMIPRAMINE TREATMENT ON THE UNCONDITIONED ALIMENTARY BEHAVIOR AND CLASSICAL CONDITIONED SALIVARY REACTIONS IN DOGS

Renard KORCZYŃSKI and Elżbieta FONBERG

Department of Neurophysiology, Nencki Institute of Experimental Biology
Warsaw, Poland

Abstract. In Experiment I the effect of imipramine treatment on the unconditioned food intake in dogs was tested. In Experiment II imipramine was injected in dogs in which classical conditioned salivary reflexes had been previously elaborated. Both conditioned and unconditioned salivation were decreased. The differentiation of conditioned salivation to CS$^+$ and CS$^-$ was disturbed during the imipramine treatment, due to the prominent decrease of conditioned salivary reflexes to CS$^+$ and also increase of reactions to CS$^-$. Imipramine treatment produced only a slight decrease of food intake. In most dogs of both groups the increase of general arousal and improvement of social contact was observed. The variability of the imipramine effect on particular parameters of alimentary behavior depended on the individual characteristics of each dog. It is concluded that in the evaluation of the effect of imipramine one should take into consideration its differential influences on various motor, autonomic and emotional, central as well as peripheral components of alimentary behavior and characteristics of the individual subjects.

INTRODUCTION

The clinical role of tricyclic antidepressants in the treatment of depressive patients is well known (see 11), whereas the effects of these drugs on animals were much less investigated. Very little is known about the effect of these drugs on various components of behavior in
normal subjects. Such investigations appeared to be very important in view of our aim to study the effect of antidepressants on depressed animals. Our previous papers (5, 6) have shown that the effect of imipramine or amitriptyline on dogs in depression caused by hypothalamic and amygdalar lesions mainly concerns the increase of general motility and sensory-motor responses. Therefore, the problem arises whether the main ameliorative effect of imipramine is not superficial i.e., that the agitation produced by activation of the sensory-motor system creates the impression of increased motivation and better mood. The excitatory action of the imipramine on the motor system is well known both from clinical observations and from neurophysiological and electrophysiological studies (9, 11, 15, 20).

It seemed important to study how the imipramine would affect the other than motor responses and, in particular, how the antidepressant treatment would change the alimentary responses, both conditioned and unconditioned. In order to exclude as much as possible the participation of the motor system in these reactions, we aimed to study in this paper the effect of imipramine on unconditioned food intake and salivary reactions. In particular salivary alimentary responses should be rather independent of the changes of the level of motor excitation.

On the other hand, antidepressants which are characterized by anticholinergic action (7) may act on parasympathetic system inhibiting it and, in consequence, produce per se decrease of salivation. Although saliva secretion depends on both sympathetic and parasympathetic mechanisms, the most fluid and abundant salivation is produced by parasympathetic activation, whereas sympathetic saliva is thick, sticky and produced in smaller amount than the parasympathetic one. Changes in salivation i.e., production of thick, scarce saliva may secondarily influence alimentary mechanisms and also produce decrease of appetite (caused by dry mouth mucose and changes in digestion), which might be falsely considered as decrease of alimentary motivation. In addition, anticholinergic effect may act centrally on alimentary system which, roughly speaking, is cholinergic (trophotropic system of Hess, 8), although biochemically it is rather complicated (12, 13, 19). This last point is very important, as in our investigation of depressive dogs we widely use alimentary responses as a detector of the level of motivation. We found that depressive dogs show a considerable decrease of food intake up to several weeks of aphagia, as well as a decrease of instrumental responses reinforced by food (3, 4, 17). Also a decrease of conditioned salivary reflexes was observed (14). In animals various alimentary reactions are often used as a test for the level of general motivational state. It seemed
therefore indispensable to study the effect of antidepressant on the alimentary responses of normal dogs. If the salivary responses and unconditioned food intake were greatly diminished in result of treatment with antidepressant, this inhibitory effect on peripheral alimentary mechanisms (2) might overshadow the excitatory effect of these drugs on the motivational level.

MATERIAL AND METHODS

The experiments were performed on 11 male mongrel naive dogs 2–3 years old, weighing 12–14 kg. The animals were housed individually in home cages about 2 × 2.5 m. Two experiments on two different groups of animals were performed.

Experiment I. Unconditioned food intake

In 5 dogs, (i) the daily total food intake, (ii) the daily time of eating and, (iii) body weight in ad lib. conditions, were registered. The food consisting of meat and cereal was presented once a day at 12 h moon. These parameters of alimentary behavior were registered throughout preimipramine, imipramine and postimipramine treatment periods, each consisting of three weeks.

Experiment II. Classical conditioned salivary reactions

In another group of 6 dogs the classical salivary reactions with differentiation were elaborated before imipramine treatment.

Salivary registration. Before the experiment started a chronic fistula of the Stensen’s duct from parotid gland was made according to the method of Ganike–Kupalov, as modified by Soltysik and Zbrożyna (18). The fistula was made unilaterally on the right side and opened on the cheek. The saliva was collected in a capsule attached to the cheek by means of Mendeleev wax. The secreted drops of saliva pushed out the water which filled the rubber tubes system, connected with electrical registration apparatus, and as saliva was secreted, drops of water were ejected and, by closing the electrical circuit, were recorded on an electrophysiograph.

Preliminary training. The training started after complete recovery from the fistula operation. The experiments were performed in sound-proof Pavlovian experimental chamber. During the first 6–12 days the dogs were adapted to the experimental conditions. At first the dogs had to adjust to the experimental chamber, to feeding from the bowls that moved automatically in the feeder placed before the dog, and to procedure of fixing of the salivary capsule onto the cheek.

Elaboration of reactions to CS+. As a positive conditioned stimulus (CS+) a sound from a generator 1,000 Hz was used, reinforced by food
(bread powder mixed with boiled minced meat). The experiments were carried out daily and each session consisted of eight trials with intertrial intervals about 2–3 min. Initially the conditioned stimulus only slightly preceded the intake of food (2–3 s); afterwards, progressively from day to day, the isolated period (CS—US interval) was prolonged to 20 s. The overlapping of CS and US was 10 s. Therefore the total duration of CS+ was 30 s (Fig. 1A).

![Fig. 1. Schematic illustration of consecutive phases of salivary recording considered during one trial of: A, positive CS+ and B, negative CS− presentation. a and a’, 10 s periods before CSI presentations; b and b’, first 10 s periods of conditioned salivations; c and c’, second 10 s periods of conditioned salivations. US, moment of food presentation, d, e, f — 10 s periods of unconditioned salivation during food consumption. CS and US overlap during 10 s (d).](image)

The positive salivary reflexes were trained before differentiation to preliminary criterion. As a criterion, we arbitrally took 10 experimental sessions, in which conditioned salivation stabilized at the level of minimum 5 drops during 10 s period of positive conditioned stimulus presentation in the second tenth of conditioned salivation i.e., preceding food reinforcement (see c Fig. 1A).

_Differentiation of positive and inhibitory_ CSI consisted in introducing a Tone of 500 Hz and 20 s duration (Fig. 1B) as a differentiated stimulus, which was never reinforced by food (CS−). This stimulus was introduced gradually during a few days from one up to four presentations of CS− interspersed randomly with four presentations of positive stimuli. The intertrial intervals were 2–3 min, as before. Training was performed until the animal reached the arbitrary criterion during 15 successive experimental sessions, which consisted of at least a double amount of drops to CS+ than to CS− — during the second 10 s period of CSI presentation (see c and c’ Fig. 1). After reaching this criterion, the imipramine was administered.

_Administration of antidepressant_. Imipramine was administered 2 h before food intake testing (Exp. I) or classical conditioning session.
(Exp. II). The drug was injected intramuscularly in doses of 1.4–1.7 mg, 2.8–3.3 mg and again 1.4–1.7 mg/kg. Each dose was given during five consecutive experimental days. The experiments were not performed on Sundays, but imipramine was injected continuously, therefore the total period of imipramine treatment was 17–18 days. In the Experiment II the procedure of imipramine treatment was repeated after 4 wk. interval.

RESULTS

General behavior. The effects of the imipramine treatments were not uniform in the 11 tested dogs. All dogs but two (Ud-2 and Sl-1) showed increased emotionality and motility. The dogs became more friendly and playful. In three dogs (Ud-2, Ud-4 and Sl-2) occurred the reduction of fear responses displayed usually by dogs in the presence of other dogs or persons, whereas in another dog (Sl-1) fearfulness arose. Three dogs (Ud-3, Ud-5 and Sl-6) during imipramine treatment displayed aggressive behavior toward persons and objects in the form of barking, attempts of attack and biting and destroying the surroundings. These fits of aggressiveness in dog Sl-6 appeared mainly when he was put in the experimental chamber and fastened in the harness. At the same time, hypersensitivity to the touch of cheek by salivary capsule, to the touch of harness and to limitation of freedom was observed. Food presentation produced decrease of aggressiveness.

During the second imipramine treatment of 6 dogs in Exp. II, the increase of positive emotionality has been observed only in two dogs (Sl-4 and Sl-5) and in two others (Sl-2 and Sl-3), on the opposite, the depression of mood has been observed. Aggression appeared in two dogs (Sl-4 and Sl-6). The dog Sl-6 displayed so strong aggressive tendencies toward the experimenter that it was impossible to continue the experiments.

The effect of imipramine treatment on general behavior was not permanent and when the administration of the drug series was accomplished, the dogs returned to their pretreatment shape of behavior. This refers also to aggressiveness, which was no longer displayed by the dogs when imipramine injections were discontinued.

Experiment I. Unconditioned food intake

As shown in Fig. 2A, the total amount of food intake decreased slightly in most dogs during imipramine administration. In one dog (Ud-5) imipramine treatment resulted in the increase of food intake, in that case, however, the increase of food intake was also noted during
the pretreatment period of feeding. The behavior of this dog was in general characterized by a greater variability of food intake in comparison to other dogs. This elevated level of food intake also continued in this dog after the imipramine treatment was accomplished. The total duration of food consumption under conditions of lib. feeding increased in three dogs (Ud-1, Ud-3 and Ud-4) during and after imipramine
injections (Fig. 2B), and thereafter returned to the level of pretreatment period. The prolonged duration of food intake as a result of imipramine treatment was significant only in two dogs (Ud-1 and Ud-3).

The increase of body weight (Fig. 2C) was slightly detained during imipramine treatment and either returned to pretreatment level or even exceeded it in all dogs after completion of drug administration.

**Experiment II. Unconditioned salivary reactions**

The first imipramine treatment produced a decrease in both unconditioned and conditioned salivation. The second imipramine treatment seemed to have even a more profound effect (Fig. 3). In five dogs the decrease of the total amount of salivation (see b, c, d, e, f, Fig. 1A) and in one dog its increase, was observed during the first imipramine treatment. After the cessation of imipramine injections the amount of salivation increased, but did not return to pretreatment level in four dogs (Fig. 4). The second application of imipramine resulted in a greater decrease of salivation in all dogs but one (Sl-5). After the second imipramine treatment also decrease of salivation was observed with the exception of one dog (Sl-6), which exhibited a violent aggressive behavior, so that the imipramine treatment was interrupted.
The separation of unconditioned and conditioned salivation (Fig. 5) shows that the first imipramine treatment produced an obvious decrease of unconditioned salivations (Fig. 5A) only in three dogs (S1-1, S1-5 and S1-6).

More prominent effect of treatment was observed on conditioned salivation to positive stimuli (Fig. 5B). In all tested dogs the conditioned salivary reactions decreased. This decrease was profound (about 40% of the pretreatment level), and after the antidepressive treatment was accomplished, it returned to the pretreatment level only in two dogs (S1-2 and S1-6).

The second application of imipramine evoked in most dogs a decrease of both unconditioned and conditioned salivation, but the latter decrease was greater. After the second imipramine treatment the salivary responses did not return to the pretreatment level. Only in dog S1-6 in which aggressive behavior occurred during imipramine treatment, and therefore the second treatment was not accomplished, the salivary reactions were recovered.

Salivary reactions to a conditioned inhibitory stimulus during first imipramine treatment (Fig. 5C) decreased only in two dogs (S1-1 and S1-5). In the first post-treatment period in four dogs (S1-1, S1-2, S1-3 and S1-6) a slight disinhibition of conditioned reactions to inhibitory stimuli was observed. The second imipramine treatment, produced a decrease of
Fig. 5. Different effects of imipramine injections on particular salivary responses. Each bar represents mean values from 60 trials (15 sessions) for individual dogs. A, drops of saliva during 10 s of unconditioned stimulus (US); B, drops of saliva during 10 s of positive conditioned stimulus (CS+); C, drops of saliva during 10 s of negative conditioned stimulus (CS–).

Salivation to CS– in most dogs, only in one dog (Sl-3) the level of salivary reactions to CS– was greater than in the initial period of observation. It was obvious that the second imipramine treatment did not double the effect of the first treatment. During the first imipramine treatment the mean percentage of decrease of salivation to the first 10 s of CS+ presentation was prominent (46.7%) in five dogs, but in respect to the second 10 s of CS+ presentation this decrease was smaller (31.6%) in six dogs.

On the other hand, the first imipramine administration produced an increase of salivation in four dogs during initial 10 s of inhibitory
stimulus (CS\(^{-}\)) (mean 25\%), but during the second 10 s salivation decreased in five dogs (mean 51.4\%).

The second imipramine treatment produced a decrease of conditioned salivation, during the first (mean 63\%) and second 10 s (mean 62\%) periods of CS\(^{+}\) presentation. In one dog (SI-1), on the contrary, the increase was observed.

Salivary responses to CS\(^{-}\) were also diminished during the first

![Diagram of conditioned stimuli ratio during differentiation in relation to pretreatment level, throughout two imipramine treatments in individual dogs. A, relation between negative and positive conditioned stimuli during the first 10 s of time obtained from CS\(^{+}\)b : (CS\(^{+}\)b + CS\(^{-}\)b'); B, the same values in respect of the second 10 s obtained from CS\(^{+}\)c : (CS\(^{+}\)c + CS\(^{-}\)c'); For denotation of b, b' and c, c' see Fig. 1. Each bar represents a mean of percent from 15 sessions.]
10 s period (mean 45%o) (5 dogs) and the second 10 s period (mean 61.5%o (4 dogs) of CS+. presentation. Dog Sl-6 was not considered because of unmanageable aggression, evoked already after a few injections of imipramine.

Figure 6 shows relations of conditioned salivation to positive and inhibitory stimuli. These relations were disturbed in all dogs, due to the diminution of salivary reactions to CS+ and in some dogs due also to the increase of salivation to CS-. In one dog (Sl-1) the differentiation of conditioned stimuli through imipramine treatment was better than before, however, after the cessation of imipramine injections the disinhibition occurred. In the same dog the second imipramine administration resulted also in the improvement of differentiation.

DISCUSSION

The results of our experiments showed that imipramine treatment produced a decrease of the salivary reaction, conditioned and unconditioned. Both the first and the second series of the antidepressive treatment produced such decrease, although the effect of the second treatment was deeper. The most evident was the imipramine effect on conditioned salivation, which fell down to about 40% of pretreatment level. Also unconditioned salivation was diminished. This effect may be explained by anticholinergic action of imipramine, which produced suppression of parasympathetic salivation.

The inhibitory effect of imipramine seems to influence selectively the salivary responses. Unconditioned food intake was almost unchanged. Also the alimentary motivation did not appear significantly influenced. The dogs were as interested in food as before the treatment, they consumed nearly the same amount of food, and only in a few dogs consumption of food was slowed down. Moreover, the effect of imipramine treatment on general reactivity and general emotional behavior of the dogs was excitatory. The dogs became more lively, more interested in their surroundings and more contactful with the experimenter. Two dogs of Exp. II became hyperreactive to tactile stimuli when put in harness in the experimental chamber, and this was followed by violent fits of aggressiveness. Two dogs of the Exp. I also became aggressive during imipramine treatment. In some dogs increase of fearfulness was also observed.

These results show that imipramine does not equally suppress in general, all observed by us behavioral parameters. It may be concluded Exp. II became hyperreactive to tactile stimuli when put in harness excitatory. This excitatory effect may be even detected in salivary
reactions. For example, in some instances an increase of salivation was observed. It mainly concerned the disinhibition of salivation to CS−, but in same dogs also the increase of conditioned salivary responses to CS+ and to unconditioned stimuli was observed in different stages of the course of salivary reflex and different periods of imipramine treatment. The most evident excitatory effect was demonstrated if we analyzed relations between salivary responses to CS+ vs. CS−, before and during imipramine treatment (Figs. 5 and 6). Due to a decrease of salivation to CS+ and in some instances a slight disinhibition of salivation to CS−, the differentiation of CS+ and CS− deteriorates during imipramine treatment. These results might suggest that although, in general, the effect of imipramine on salivary reactions is inhibitory, the general excitatory influence of this drug may reflect also in release from inhibition of the previously inhibited responses to CS−. The changes in differentiation may also reflect the disturbances in inhibitory — excitatory balance and interactions between central excitatory effect of imipramine and its peripheral inhibitory influence on the salivary responses. It may be also suggested that imipramine, acting both centrally and peripherally through parasympathetic system on salivary responses, suppresses them selectively, whereas in general its action is excitatory. Those two mechanisms may counteract each other. The disinhibition of salivary responses to CS− and the episodes of increased positive salivary-conditioned as well as unconditioned-reactions, which in particular dogs are observed in various stages of imipramine treatment and in various parameters of the experiments, may be considered as indications of the excitatory effect of imipramine treatment. On the other hand, the fact that in some dogs the imipramine treatment produced an increase of salivation instead of decrease (which was a rule), may be explained by individual characteristics of the dogs, and their different susceptibility to drug treatment. Such individual variability may indicate that the neurochemical characteristics of normal dogs differ to each other. These differences are probably enhanced after various hypothalamic and amygdalar lesions, as these structures are strongly involved in the mechanisms of adrenergic-cholinergic relations. The adrenergic-cholinergic balance is probably different in different in fact subjects as well as in operated dogs after different lesions. Also the susceptibility to certain drugs may vary, depending on different metabolic rates and biochemical turnover. The differential effect of the tricyclic antidepressants on various physiological mechanisms underlying behavioral symptoms should be also taken into consideration. It may depend on the role of cholinergic, adrenergic and
serotonergic systems in particular responses. An anticholinergic action of imipramine may explain the decrease of salivation.

Whereas its adrenergic action may produce activation of some behavioral components, including aggression (16), the action on the serotonergic receptors may, on the other hand, be responsible for some depressive effects (1).

The problem arises why the effect of imipramine is greater on conditioned vs. unconditioned salivation, if imipramine would suppress parasympathetic salivary mechanisms selectively. It is possible that the observed changes in conditioned salivation reveal changes in central autonomic mechanisms (a signalizing role of CS for the salivary excretion), whereas changes of unconditioned salivation reveal disturbances of peripheral autonomic mechanisms (reflex from mouth mucose tactile and taste endings). In this case our results might be explained by greater effect of imipramine on central parasympathetic mechanisms.

On the other hand, imipramine may disturb the signalizing role of CS as such. From clinical observations it is known that during imipramine treatment the discrimination and perception of sensory stimuli decrease. For example, before imipramine treatment a patient was able to perceive separately light flashes presented close to each other at certain rates, whereas during an antidepressive drug treatment the separate flashes were perceived as one continuous visual stimulus. In order to enable the discrimination of particular flashes, the intervals between them had to be elongated. Psychiatrists explain this phenomenon by a decreased state of wakefulness, caused by anticholinergic action of antidepressive drugs (10). This may be explained by electrophysiological data which show that tricyclic antidepressants produce a synchronization of cortical potentials and an increase of the threshold of reticular formation for neocortical activation (20). In our experiments the decrease of conditioned salivation and the changes of relations between \( CS^+ \) and \( CS^- \) might be also explained by disturbances in the exteroceptive signalizing role of the conditioned stimuli. The fact that the most prominent effect of imipramine on differentiation is noted during the first 10 s of CSi presentation in the first series of imipramine treatment support such suggestion.

The selective inhibitory effect of imipramine on the salivary responses makes the salivary procedure not useful as a test in the investigation of motivational changes produced by antidepressive treatment. In addition the decrease of salivation, producing a feeling of dry mouth and a deteriorating digestion of carbohydrates, may secondarily influence the appetite. Such procedure, however, seems useful as an additional control test to the study of basic features of the action of
antidepressive drugs on central vs. peripheral mechanisms of CNS. Our present experiments performed on normal dogs seem to be important in such respect that they fill the gap in investigations of the role of imipramine in various basic physiological mechanisms in normal subjects. Present results point out to the complexity and variability of the features of imipramine effect on various explored parameters of behavior and on individual subjects. Such prominent differences in the sensitivity to imipramine treatment in dogs are interesting per se and need a further, more detailed analysis. Our present results did not resolve the basic question, concerning the physiological mechanisms of imipramine action and why its role in the depressive syndrome is ameliorating and useful as an essential remedy in this illness. All these problems need further investigations and the experiments on the effects of imipramine as well as other antidepressants on the various behavioral aspects are already in progress.

We are greatly indebted to Dr. K. Jaworska for her valuable comments, and Mrs. J. Krakowska and Mrs. A. Kurzaj for their technical assistance. This investigation was supported by Project 10.4.1.01 of the Polish Academy of Sciences.

REFERENCES


Accepted 16 February 1979

Renard KORCZYŃSKI and Elżbieta FONBERG, Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland.