Br. J. Pharmac. Chemother. (1966), **27**, 468–478.

A NEUROPHARMACOLOGICAL STUDY OF THE ANTIDEPRESSANT ACTION OF IMIPRAMINE

BY

K. P. BHARGAVA, J. N. SINHA AND K. K. TANGRI

From the Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow, India

(*Received April* 29, 1966)

Imipramine is chemically similar to chlorpromazine, and like chlorpromazine it produces motor retardation, prolongation of barbiturate-induced sleeping time and synchronization of the spontaneous corticogram (Domenjoz & Theobald, 1959; Sigg, 1959, 1962); but unlike chlorpromazine it can reverse reserpine-induced sedation (Sulser, Watts & Brodie, 1962) and potentiate the effects of catecholamines (Osborne & Sigg, 1960) and amphetamine (Stein & Seifter, 1961a, b). The antidepressant properties of imipramine were first discovered after prolonged administration of the drug to patients (Kuhn, 1957, 1958). Electrophysiological studies with imipramine have not provided a satisfactory explanation for the antidepressant effect (Schallek & Kuehn, 1960; Penaloza-Rojas, Bach-Y-Rita, Rubio-Chevannier & Hernandez-Peon, 1961; Stein & Seifter, 1961a, b; Rubio-Chevannier, Bach-Y-Rita, Penaloza-Rojas & Hernandez-Peon, 1961).

The present work was undertaken to study the effects of imipramine on the electrocortical arousal induced by mid-brain reticular stimulation in *cerveau isolé* cats, on the excitability of cortical neurones in the neuronally isolated cerebral cortical slab preparation of dogs, and on the integration of somatic reflexes at the spinal and supraspinal levels in intact and spinal transected cats.

A preliminary report was presented at the International CNS Drugs Symposium at Hyderabad (India) in January, 1966.

METHODS

Electroencephalographic studies

Electrocortical arousal due to mid-brain reticular stimulation was studied in *cerveau isolé* cats by the technique already described (Saxena, Tangri & Bhargava, 1964). The cortical slab of dogs was neuronally isolated by the method first described by Kristiansen & Courtois (1949) and subsequently used by us (Sinha, Tangri & Bhargava, 1966). All drugs were given intravenously.

Somatic reflex studies in cats

The studies on somatic reflexes were carried out in intact cats under light chloralose anaesthesia (50 mg/kg, intravenously) and in spinal (C7) transected cats. The patellar monosynaptic reflex was elicited by the technique of Calma & Wright (1947). Polysynaptic facilitation of the patellar reflex was obtained by stimulation of lateral facilitatory area of the reticular formation and by contralateral sciatic nerve stimulation. Polysynaptic inhibition of the patellar reflex was obtained by stimulation of the ventromedial inhibitory area of the reticular formation and by contralateral sciatic nerve stimulation. Monosynaptic inhibition of the patellar reflex was elicited by ipselateral sciatic nerve

stimulation. These procedures have been described in earlier communications (Bhargava & Srivastava, 1964, 1965a).

The linguomandibular reflex was elicited by the method of King & Unna (1954), as suitably modified by Bhargava & Srivastava (1965b).

RESULTS

Electroencephalographic studies

Effect of imipramine on reticular " arousal " in cerveau isolé *cats.* Imipramine (0.1 to 4 mg/kg) was tested on the spontaneous and evoked electrocortical activities in six *cerveau isolé* cats. In all the doses used imipramine increased the neuronal synchronization and the rate of spontaneous spindles. Also, the threshold of electrocortical arousal elicited by mid-brain reticular stimulation was raised. Similar findings have been reported by Van Meter, Owens & Himwich (1960) and Vernier (1961).

Effect of imipramine on the spontaneous and evoked activity of the neuronally isolated cortex of dogs. Imipramine (0.1 to 8 mg/kg) induced high voltage slow wave activity initially in both the isolated and the intact cerebral cortices in all the doses used in twelve dogs. Also, it increased the threshold and decreased the duration of the evoked after-discharge in the isolated cortex. However, a delayed " stimulant " effect characterized by a desynchronized pattern in the intact cortex and decreased threshold and increased duration of after-discharge in the isolated cortex was observed only with high doses of imipramine after a period of 6 hr. Typical results of such a study are shown in Fig. 1.

Effect of desmethylimipramine on reticular " arousal " in cerveau isolé *cats.* In order to account for the delayed stimulant effect of imipramine in the encephalographic studies, it was thought desirable to study the effects of desmethylimipramine, an important

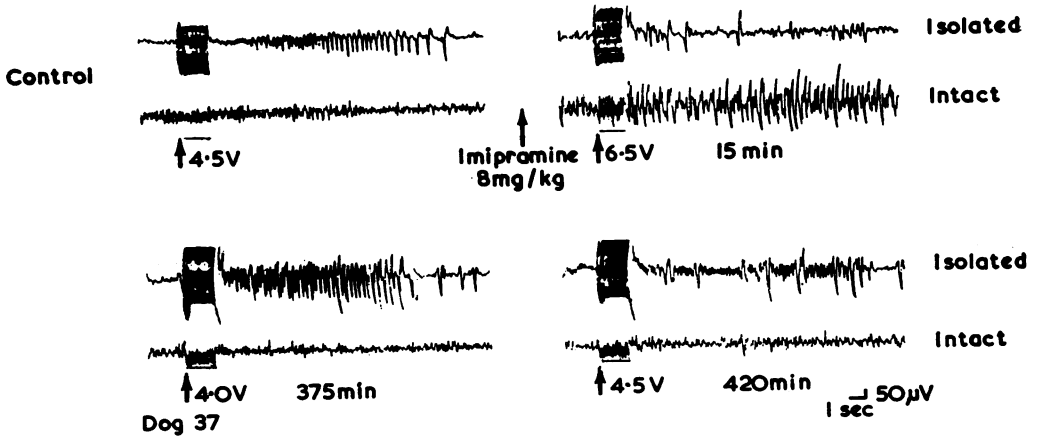

Fig. 1. Records showing the effects of imipramine (8 mg/kg, intravenously) on the spontaneous activities of the intact and the isolated cerebral cortices. Note that imipramine induced high voltage slow wave activity in the corticograms 15 min after it was administered ; this lasted for 6 hr. The threshold of the after-discharge is raised from 4.5 V in the control to 6.5 V after 15 min. At 375 min it induced low voltage fast wave activity in both intact and isolated cerebral cortices. Also, the threshold was lowered to 4 V (control 4.5 V). Recovery occurred in 420 min.

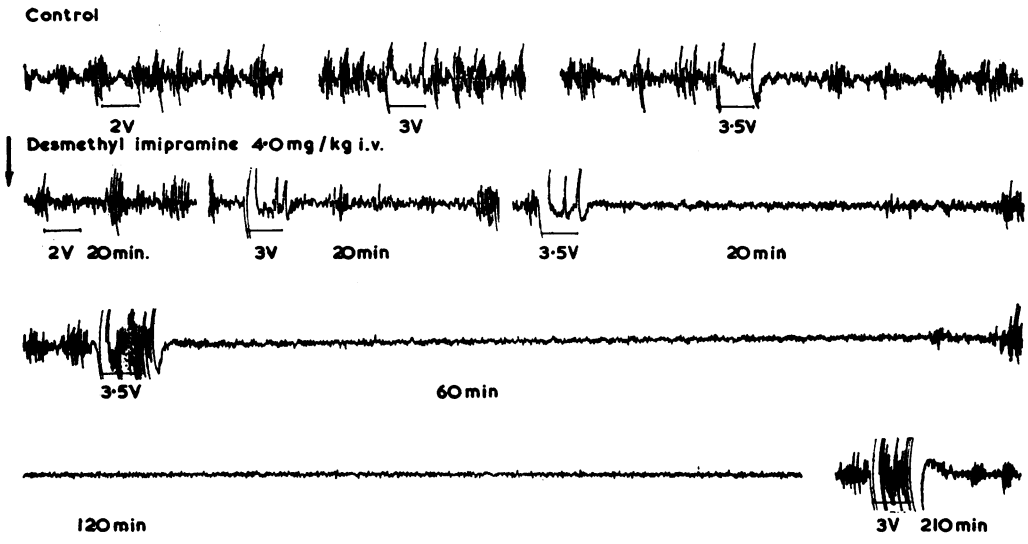

Fig. 2. Records showing the effect of desmethylimipramine (4 mg/kg, intravenously) on the reticular arousal in a *cerveau isolé* cat. Note that desmethylimipramine decreased the spontaneous synchronization and the rate of spindles; it lowered the threshold from 3.5 V in the control to 2 V after 20 min. Also, the duration of the reticular arousal was markedly increased. Recovery seen after 210 min.

metabolite of imipramine. Desmethylimipramine (2 to 8 mg/kg) decreased the neuronal synchronization and the rate of spontaneous spindles in six *cerveau isolé* cats. The threshold of reticular arousal was lowered and the duration of the arousal response was markedly increased. Recovery from the drug effect occurred in approximately 3.75 hr. Figure 2 shows a typical effect of desmethylimipramine (4 mg/kg).

Effect of desmethylimipramine on the spontaneous and evoked activity of the neuronally isolated cortex of dogs. Desmethylimipramine (2 to 8 mg/kg) initially induced high voltage slow wave activity in the intact and isolated cortex in six dogs. There was no change in the threshold or the duration of the after-discharge. However, a stimulant effect was clearly observed after 2.5 hr with 4 mg/kg and after 1.5 hr with 8 mg/kg. At the peak stimulant phase the intact cortex exhibited low voltage fast wave activity, while in the isolated cortex the threshold of the after-discharge was lowered and the duration was markedly increased. The stimulant effect lasted from 200 to 250 min depending upon the dose. Figure 3 shows the effects of desmethylimipramine (8 mg/kg).

Somatic reflex studies

Effect of imipramine of the patellar reflex. The patellar reflex was elicited in spinal (C7) transected cats. Intrathecal injection of imipramine (1 to 2 mg) consistently depressed the amplitude of the patellar reflex. The inhibitory effect of imipramine appeared within 5 to 7 min, reached its peak in 10 to 15 min and lasted up to 75 to 90 min depending on the dose. Doses less than 1 mg did not inhibit the patellar responses.

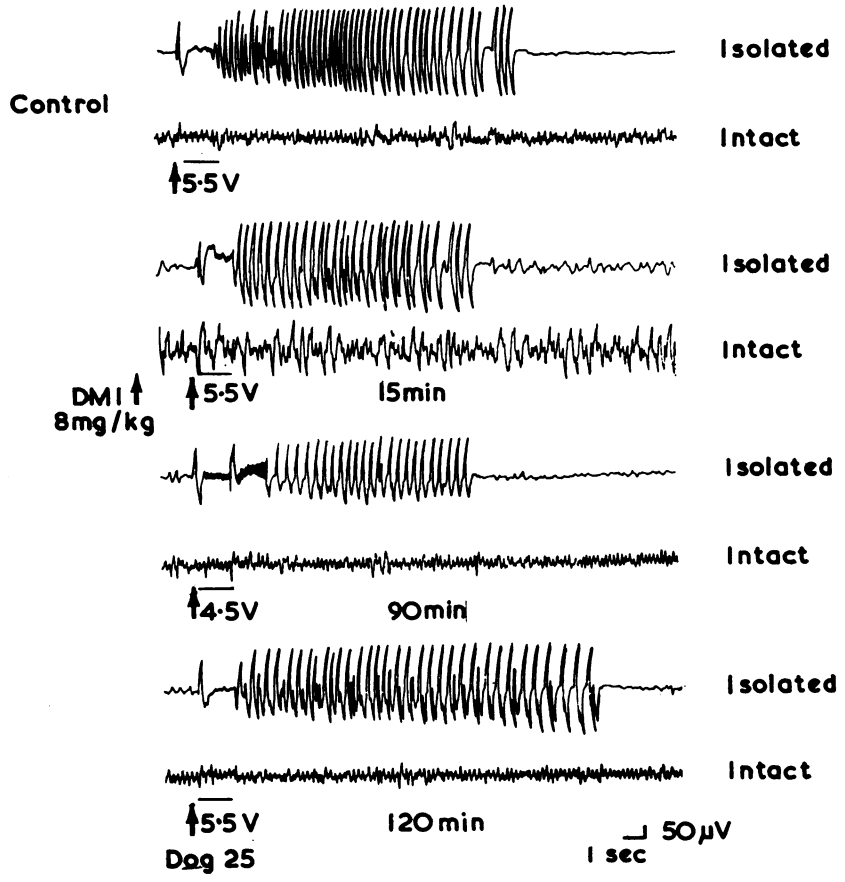

Fig. 3. Records showing the effect of intravenous desmethylimipramine (8 mg/kg) on the spontaneous activities of the intact and isolated cortices and on the threshold of the after discharge in the isolated cerebral cortex. Note that desmethylimipramine induced high voltage slow wave activity in both the cortices (15 min). However, the threshold of the after discharge was unaffected at 15 min. At 90 min, the drug induced low voltage fast wave activity in the corticograms and lowered the threshold of the after discharge from 5.5 V in the control to 4.5 V. Recovery seen at 120 min.

Effect of imipramine on facilitation of the patellar reflex induced by stimulation of the facilitatory area of the reticular formation. The effect of imipramine was studied on the facilitation of the patellar reflex induced by stimulation (3 to 5 V, 120 shocks/sec for 15 sec) of the facilitatory area of the reticular formation in three cats. Stimulation of the reticular facilitatory area also elicited a pressor response. Typical effects of topical application of imipramine (1.25%) on the amplitude of the patellar reflex and on its facilitation are shown in Fig. 4. Local application of the drug (soaked in a cotton pledget) at the site of electrode penetration reduced the amplitude of the patellar responses and abolished the facilitation of the patellar reflex to reticular stimulation. The pressor response to the central stimulation was also depressed by imipramine. Only partial recovery of the responses was observed at 90 min.

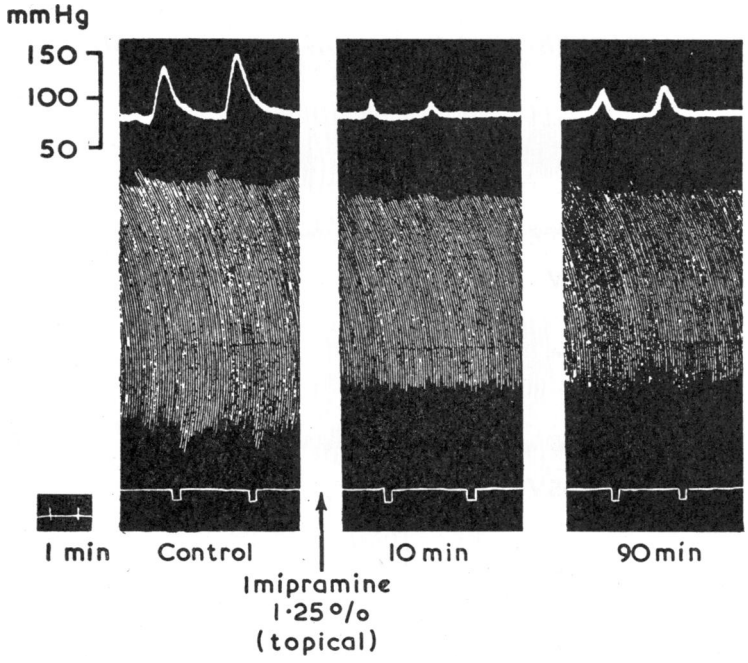

Fig. 4. Records showing blood pressure (upper tracing), patellar tap responses every 5 sec (lower tracing) and polysynaptic facilitation of the patellar reflex due to reticular formation stimulation (3 V, 120 shock/sec for 15 sec). Note that topical application of imipramine (1.25%) reduced the amplitude of the patellar reflex and abolished the reticular facilitation of the patellar reflex 10 min after the drug was administered. Recovery from the drug effect occurred in 90 min.

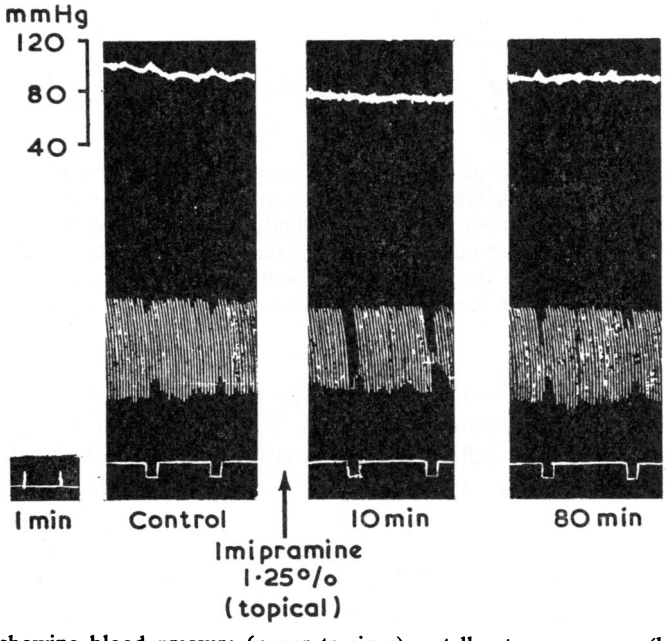

Fig. 5. Records showing blood pressure (upper tracings), patellar tap responses (lower tracing) and polysynaptic inhibition of the patellar reflex due to reticular formation stimulation (0.5 V, 120 shocks/sec for 15 sec). Note reduction in the amplitude of the patellar reflex and enhancement of the inhibition of the patellar reflex to reticular stimulation, 10 min after the topical application of imipramine (1.25%). Recovery from the drug effect was obvious at 80 min.

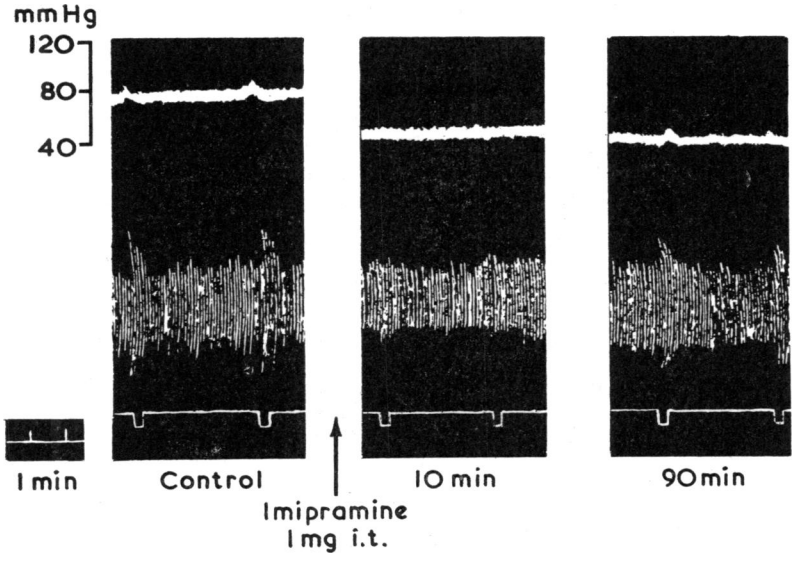

Fig. 6. Records showing blood pressure (upper tracing), patellar tap responses (lower tracing) and polysynaptic facilitation of the patellar reflex due to contralateral sciatic nerve stimulation (3 V, 120 shocks/sec for 15 sec). Intrathecal imipramine (1 mg) reduced the amplitude of the patellar reflex and abolished the facilitation to nerve stimulation (see at 10 min). Recovery occurred at 90 min.

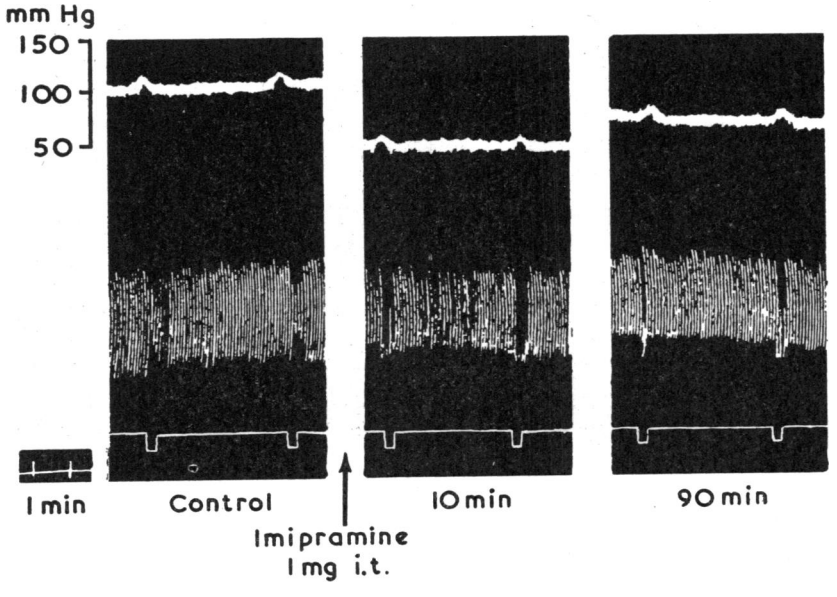

Fig. 7. Records showing blood pressure (upper tracing), patellar reflex (lower tracing) and polysynaptic inhibition of patellar reflex due to contralateral sciatic nerve stimulation (10 V, 120 shocks/sec for 15 sec). Note that intrathecal imipramine (1 mg) depressed the patellar tap responses and increased the inhibition to nerve stimulation. Recovery seen at 90 min.

Effect of imipramine on inhibition of the patellar reflex induced by stimulation of the inhibitory area of the reticular formation. Polysynaptic inhibition of the patellar reflex was elicited by stimulating the inhibitory area of the brain stem reticular formation by means of rectangular pulses (0.25 to 0.5 V, 120 shocks/sec for 15 sec) in three cats. Topical application of imipramine (1.25%) reduced the amplitude of the patellar responses and increased the reticular inhibition of the patellar reflex. Recovery from the drug effect occurred in 80 min. The results of one such experiment are shown in Fig. 5.

Effect of imipramine on facilitation of patellar reflex due to contralateral sciatic nerve stimulation. Polysynaptic facilitation of the patellar reflex due to contralateral sciatic nerve stimulation (3 V, 120 shocks/sec for 15 sec) was elicited in four spinal (C7) transected cats, and the effect of imipramine (0.5 to 2 mg) was studied. Figure 6 shows the results of one such experiment where intrathecal injection of imipramine (1 mg) reduced the amplitude of the patellar reflex and abolished the facilitation of the patellar reflex due to nerve stimulation (see at 10 min). However, the recovery of the patellar reflex and its facilitation occurred after 1.5 hr.

Effect of imipramine on inhibition of patellar reflex due to contralateral sciatic nerve stimulation. Polysynaptic inhibition of the patellar reflex was elicited by contralateral sciatic nerve stimulation (10 V, 120 shocks/sec for 15 sec) in three spinal (C7) transected

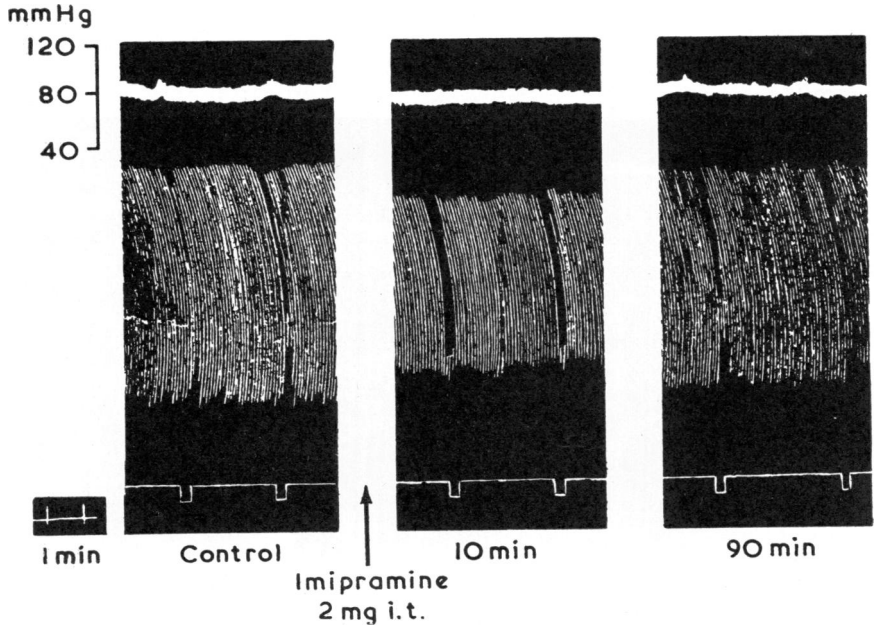

Fig. 8. Records showing the effect of intrathecal imipramine (2 mg) on the blood pressure (upper tracing), patellar tap responses (lower tracing) and monosynaptic inhibition of the patellar tap responses elicited by the stimulation of ipselateral sciatic nerve (0.5 V, 100 shocks/sec for 15 sec). Note that the drug reduced the amplitude of the patellar reflex and increased the inhibition to nerve stimulation. Recovery occurred at 90 min.

cats. In Fig. 7 are shown the results of one such experiment. Intrathecal injection of imipramine (1 mg) reduced the amplitude of the patellar reflex and enhanced the inhibition of the patellar reflex to nerve stimulation. Recovery was observed in 1.5 hr.

Effect of imipramine on inhibition of patellar reflex due to ipselateral sciatic nerve stimulation. The effect of imipramine was studied on the monosynaptic inhibition of the patellar reflex due to ipselateral sciatic nerve stimulation (0.3 to 1 V, 100 shocks/sec for 15 sec) in three spinal cats. Intrathecal imipramine (1 to 2 mg) consistently reduced the amplitude and enhanced the inhibition of the patellar reflex due to nerve stimulation. Recovery from such inhibitory effects occurred in about 1.5 hr. Figure 8 shows the records of one such experiment.

Effect of intravenous imipramine on the linguomandibular and patellar reflexes. The effects of intravenous imipramine were observed simultaneously on the linguomandibular reflex and the patellar reflex in three cats. Figure 9 shows the results of one such study. An intravenous injection of imipramine (2 mg/kg) depressed the linguomandibular reflex without affecting the patellar responses. Thus, the polysynaptic reflex integrated at the brain stem level was selectively depressed by imipramine.

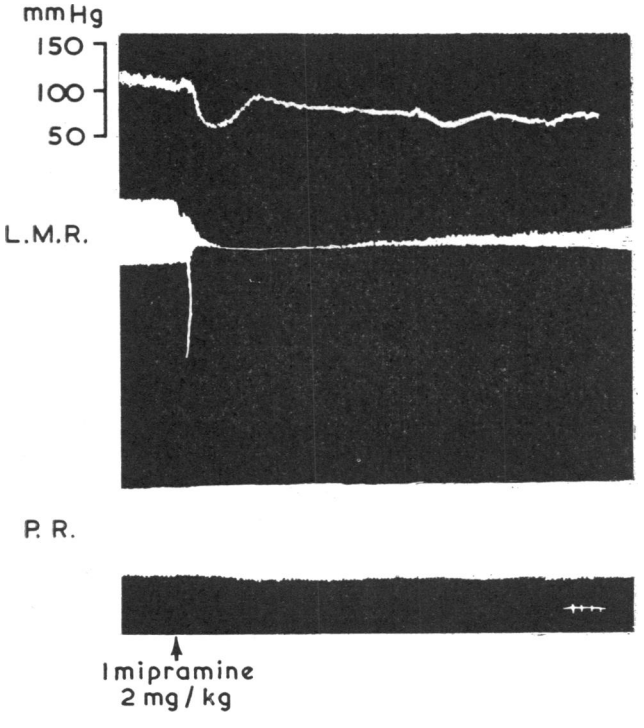

Fig. 9. Records showing the effect of intravenous imipramine (2 mg/kg) simultaneously on blood pressure (upper tracing), polysynaptic linguomandibular reflex (LMR, middle tracing) and monosynaptic patellar reflex (PR, lower tracing). Note that imipramine depressed the linguomandibular reflex without affecting the patellar reflex.

DISCUSSION

The precise mechanism of the antidepressant action of imipramine is not clear. A number of hypotheses have been put forward to explain this action. Schallek & Kuehn (1960) and Schallek, Kuehn & Jew (1962) suggested that the stimulant action of imipramine on the psyche is due to its depressant effect on the septum. However, the observation that the activity of the septum was also depressed by meprobamate, a tranquillizer, did not favour this contention. That low doses of imipramine sensitize the hypothalamus to produce a rage reaction only in low doses was suggested by Penaloza-Rojas *et al.* (1961) to account for its antidepressant action. However, these investigators observed a depression of the hypothalamic responses with high doses of imipramine. Stein & Seifter (1961a, b) showed augmentation of amphetamine-induced potentiation of hypothalamic self-stimulation responses with low doses of imipramine and a block of these responses with high doses. Rubio-Chevannier *et al.* (1961) suggested that lowering of the threshold of sensory as well as reticular arousal with low doses of imipramine was responsible for the antidepressant activity. However, a rise in the threshold of sensory as well as reticular arousal was observed by Van Meter *et al.* (1960) and by Vernier (1961) with low as well as high doses of imipramine. On the basis of the antagonism of reserpine-induced sedation, Sulser, Bickel & Brodie (1964) suggested that the antidepressant action of imipramine may be due to its conversion into desmethylimipramine in the body. So far a satisfactory explanation for the psychostimulant action of imipramine is not available.

The present study was undertaken with a view to investigating the effects of imipramine on the isolated cerebral cortical neurones, the ascending reticular activating system and the descending motor system. From our results it is clear that imipramine, in all the doses used, exerted a depressant effect on the mid-brain reticular arousal system and depressed the electrical excitability of both intact as well as isolated cortical neurones. The most significant finding was the delayed stimulant effect of imipramine (8 mg/kg) after a period of 375 min on the cortical neurones (see Fig. 1). In order to account for the delayed stimulant action of imipramine the effect of desmethylimipramine was observed under similar experimental conditions. In contrast to imipramine, desmethylimipramine had a stimulant action on the reticular activating system and the neuronal excitability of the isolated cerebral cortex (see Figs. 2 and 3). It may be noted that only high doses (8 mg/kg) of imipramine elicited the delayed stimulant actions. The stimulant action of desmethylimipramine was, however, seen with lower doses (4 mg/kg) and was quick in onset. The delayed stimulant action of imipramine on the sensory cortical neurones is probably due to its metabolic transformation into desmethylimipramine.

The reserpine-induced depressive syndrome and the clinical state of endogenous depression are both characterized by increased muscular tone, which is antagonized by the antidepressive agents (Sulser *et al.*, 1962, 1964). Imipramine and allied group of drugs possess a central muscle relaxant property (Sinha, Srimal, Dixit, Chandra & Bhargava, 1966). Therefore, the action of imipramine was studied on the central integration of somatic reflexes. Imipramine depressed the motor system at the level of the descending reticular formation and the spinal motor neurones. Such a central muscle relaxant action of imipramine contributes to the beneficial effect observed in endogenous depression.

The results of the present study clearly demonstrate the inhibitory action of imipramine on the ascending reticular activating system, the cortical neurones *per se*, the descending brain-stem facilitatory and inhibitory neurones of the reticular formation concerned with motor function and the spinal motor neurones. This inhibitory action of imipramine at all levels of the neuraxis appears to be similar to that of the phenothiazine group of tranquillizers, to which imipramine is chemically closely related. Thus, imipramine primarily appears to be a tranquillizer like the phenothiazines, and in the body the tranquillizing action is converted into the characteristic antidepressant action due to the accumulation of an active metabolite, desmethylimipramine. In the present study the stimulant action of desmethylimipramine on the ascending reticular activating system and the neuronally isolated cerebral cortex has been clearly demonstrated. The sensitizing action of desmethylimipramine on the sensory cortex, concerned with the control of the psyche, may be the most important factor in the antidepressant activity of the imipramine group of drugs.

SUMMARY

1. The effect of imipramine has been studied on the electrocortical arousal induced by mid-brain reticular stimulation (*cerveau isolé* cats), the excitability of the cortical neurones in isolated cerebral cortical slab preparations (dogs) and the central integration of somatic reflexes (intact and spinal cats).

2. Imipramine inhibited the ascending reticular activating system as well as the neuronal excitability of the cortical neurones.

3. A clear-cut stimulant action was observed on the cortical neurones with imipramine after a delay of about 6 hr.

4. A stimulant action of quicker onset was seen with doses of desmethylimipramine lower than those of imipramine under identical experimental conditions.

5. The delayed stimulant action of imipramine could be the result of its metabolic transformation into an active metabolite, for example, desmethylimipramine.

6. Imipramine exerted a potent inhibitory effect on the central integration of the somatic reflexes both at spinal and supraspinal levels.

7. The sensitizing action of desmethylimipramine on the sensory cortex may be responsible for the antidepressant effect of the imipramine group of drugs.

The authors wish to thank the Council of Scientific and Industrial Research for financial assistance. We are grateful to Dr E. R. De Fries for the supply of imipramine and desmethylimipramine used in this study. Thanks are also due to our colleagues Drs P. N. Saxena and B. P. Jaju for valuable suggestions.

REFERENCES

BHARGAVA, K. P. & SRIVASTAVA, R. K. (1964). Nonspecific depressant action of γ-aminobutyric acid on somatic reflexes. *Br. J. Pharmac. Chemother.*, **23**, 391–398.

BHARGAVA, K. P. & SRIVASTAVA, R. K. (1965a). Central effects of haloperidol on somatic reflexes. *Br. J. Pharmac. Chemother.*, **25**, 751–757.

BHARGAVA, K. P. & SRIVASTAVA, R. K. (1965b). Antitetanus activity of central muscle relaxants. *Br. J. Pharmac. Chemother.*, **25**, 74–80.

CALMA, I. & WRIGHT, S. (1947). Effects of intrathecal injection of KCl and other solutions in cats. Excitatory action of K ions on posterior nerve root fibres. *J. Physiol., Lond.*, **106**, 211–235.

DOMENJOZ, R. & THEOBALD, W. (1959). Zur Pharmakologie des Tofranil (N-(3-Dimethylaminopropyl)-iminodibenzyl-Hydrochlorid). *Archs int. Pharmacodyn. Thér.*, **120**, 450–489.

KING, E. E. & UNNA, K. R. (1954). The action of mephenesin and other interneuron depressants on the brain stem. *J. Pharmac. exp. Ther.*, **111**, 293–301.

KRISTIANSEN, K. & COURTOIS, G. (1949). Rhythmic electrical activity from isolated cerebral cortex. *Electroen. Neurophysiol.*, **1**, 265–272.

KUHN, R. (1957). Uber die Behandlung depressiver Zustaende mit einem Iminodibenylderivat (G 22355). *Schweiz. med. Wschr.*, **87**, 1135–1140.

KUHN, R. (1958). The treatment of depressive states with G 22355 (imipramine hydrochloride). *Am. J. Psychiat.*, **115**, 459–464.

OSBORNE, M. & SIGG, E. B. (1960). Effects of imipramine on the peripheral autonomic system. *Archs int. Pharmacodyn. Thér.*, **129**, 273–289.

PENALOZA-ROJAS, J. H., BACH-Y-RITA, G., RUBIO-CHEVANNIER, H. F. & HERNANDEZ-PEON, R. (1961). Effects of imipramine upon hypothalamic and amygdaloid excitability. *Expl. Neurol.*, **4**, 205–213.

RUBIO-CHEVANNIER, H. F., BACH-Y-RITA, G., PENALOZA-ROJAS, J. H. & HERNANDEZ-PEON, R. (1961). Potentiating action of imipramine upon reticular arousal. *Expl. Neurol.*, **4**, 214–220.

SAXENA, P. N., TANGRI, K. K. & BHARGAVA, K. P. (1964). An experimental study of the synchronizing system in the brain. *Electroen. Neurophysiol.*, **17**, 506–512.

SCHALLEK, W. & KUEHN, A. (1960). Effects of psychotropic drugs on limbic system of cat. *Proc. Soc. exp. Biol. Med.*, **105**, 115–117.

SCHALLEK, W., KUEHN, A. & JEW, N. (1962). Effects of chlordiazepoxide (librium) and other psychotropic agents on the limbic system of the brain. *Ann. N.Y. Acad. Sci.*, **96**, 303–314.

SIGG, E. B. (1959). Pharmacological studies with Tofranil. *Can. psychiat. Ass. J.*, **4** (Suppl.), 575–585.

SIGG, E. B. (1962). The pharmacodynamics of imipramine. In *Psychosomatic Medicine, First Hahnemann Symposium*, ed. NODINE, J. H. & MOYER, J. H., pp. 671–678. London: Kimpton.

SINHA, J. N., SRIMAL, R. C., DIXIT, K. S., CHANDRA, O. & BHARGAVA, K. P. (1966). Central muscle relaxant activity of imipramine, amitriptyline and desmethylimipramine. *Jap. J. Pharmac.*, **16**, 120–122.

SINHA, J. N., TANGRI, K. K. & BHARGAVA, K. P. (1966). Effect of β-phenylisopropyl hydrazine (JB 516) on the isolated cerebral cortex. *Electroen. Neurophysiol.*, **21**, 301-303.

STEIN, L. & SEIFTER, J. (1961a). Possible mode of antidepressive action of imipramine. *Science, N.Y.*, **134**, 286–287.

STEIN, L. & SEIFTER, J. (1961b). Imipramine, chlorpromazine and amphetamine interactions; possible mode of anti-depressive action of imipramine. *Fedn Proc.*, **20**, 395.

SULSER, F., BICKEL, M. H. & BRODIE, B. B. (1964). The action of desmethylimipramine in counteracting sedation and cholinergic effects of reserpine-like drugs. *J. Pharmac. exp. Ther.*, **144**, 321–330.

SULSER, F., WATTS, J. & BRODIE, B. B. (1962). On the mechanism of antidepressant action of imipramine-like drugs. *Ann. N.Y. Acad. Sci.*, **96**, 279–286.

VAN METER, W. G., OWENS, H. F. & HIMWICH, H. E. (1960). Effects on rabbit brain of a new drug with psychotomimetic properties. *J. Neuropsychiat.*, **1**, 129–134.

VERNIER, V. G. (1961). The pharmacology of antidepressant agents. *Dis. nerv. Syst.*, **22**, suppl. May, 7–13.