SUMMARY
SUMMARY AND CONCLUSION

CLONIDINE-INDUCED BEHAVIOURAL DESPAIR: AN EXPERIMENTAL MODEL TO DETECT THE ANTIDEPRESSANT ACTIVITY

The effects of various alpha\textsubscript{2} adrenoceptor agonists on forced swimming-induced despair behaviour were studied in mice. Clonidine, B-HT 920 and guanfacine significantly prolonged the total immobility duration. Clonidine-induced behavioural despair was antagonized by prior treatment with yohimbine. The tricyclic antidepressants, viz. imipramine, desipramine, trimipramine, amitriptyline, nortriptyline and doxepine, the MAO inhibitor tranylcypromine, and the psychostimulant amphetamine reversed clonidine-induced behavioural despair. Chronic treatment with imipramine evoked more pronounced reversal as compared to acute treatment. Diazepam, a skeletal muscle relaxant did not modify the clonidine effect. On the other hand, adenosine showed potentiation of the submaximal response of clonidine. Clonidine-induced enhancement in immobility
duration was not affected by the variation in water temperature from 22° to 40°C. These observations suggest that clonidine-induced behavioural despair is probably mediated through its presynaptic action on alpha_2 adrenoceptors resulting in reduced central noradrenergic outflow. The present data proposes a simple test system to induce depression-like syndrome in animals, sensitive to antidepressant therapy.

BETA ADRENOCEPTOR INVOLVEMENT IN FORCED SWIMMING-INDUCED IMMOBILITY OF MICE

Modulation of forced swimming-induced immobility by β-adrenoceptor activation was investigated in mice. Isoprenaline prolonged the immobility duration of mice in a dose-related manner, when the animals were forced to swim for 6 min period. On the other hand, salbutamol (a β_2-agonist) reduced the immobility duration of mice. Pretreatment with propranolol (1, 2, 4, 8 and 16 mg/kg, ip) atenolol (10 mg/kg, ip) and metoprolol (10 mg/kg, ip) antagonized the immobility-enhancing effect of isoprenaline. Chronic administration (10 mg/kg/day) for 8 days of propranolol and imipramine protected the animals from isoprenaline-evoked prolongation of immobility. These findings
suggest that central $\beta_1$- and $\beta_2$-subtypes of adrenoceptors may be acting in opposite directions to modify the immobility duration of mice. Activation of central $\beta_1$-adrenoceptors may lead to enhanced behavioural despair whereas, a reverse effect may be observed on $\beta_2$-adrenoceptor activation.

MODIFICATION BY TRICYCLIC ANTIDEPRESSANTS OF CORTICAL EEG CHANGES INDUCED BY CLONIDINE IN RATS

The effects of various tricyclic antidepressants on clonidine-induced electroencephalographic changes were investigated in rats. The EEG pattern of conscious rats was recorded by means of bipolar electrodes, implanted chronically. Clonidine (50, 150 and 300 $\mu$g/kg) not only synchronized cortical EEG pattern but also evoked signs of behavioural depression within 15 min of its administration. Pre-treatment with imipramine, desipramine, trimipramine, amitriptyline, nortriptyline and doxepin reduced clonidine-induced EEG synchrony without showing any effect per se. Acute treatment with tricyclic antidepressants failed to modify but, chronic treatment abolished the clonidine-induced behavioural depressive signs. Chronic administration of tricyclic antidepressants (10 mg/kg/day) evoked more pronounced anta-
gonism of the EEG effects of clonidine. Yohimbine (200 μg/kg) pretreatment inhibited both, clonidine-induced EEG synchrony and behavioural effects. Guanfacine as well as B-HT 920, elicited clonidine-like effects on cortical EEG pattern and behaviour. The present data suggest that the antagonism of clonidine-induced EEG synchronization in conscious animals could serve as a useful test for screening of agents with antidepressant potential.

ROLE OF ALPHA₂ ADRENOCEPTORS IN ANTINOCICEPTION

The involvement of alpha₂ adrenoceptors in antinociception was investigated and apparent pA₂ values determined using the tail flick technique in mice. Among various alpha₂ adrenoceptor agonists employed (viz. clonidine, guanfacine and B-HT 920) only clonidine exhibited antinociception in a dose-dependent manner, an effect analogous to morphine. B-HT 920 and guanfacine failed to show increased antinociception, with corresponding increase in concentration. Yohimbine (1.28x10⁻⁸ - 2.56x10⁻⁷ M/kg) and naloxone (2.75x10⁻⁸ - 2.2x10⁻⁷ M/kg) antagonized the antinociceptive effect of clonidine and morphine, respectively. Higher concentration of yohimbine was required to block the action of morphine. However, naloxone (2.75x10⁻⁷ M/kg) did not block the antino-
The receptive effect of clonidine. In vivo equivalents of $pA_2$ values for morphine-naloxone and clonidine-yohimbine pairs were calculated and found to be 7.09 and 7.45, respectively. The probable involvement of a common antinociceptive site for the observed phenomenon is discussed.

Naloxone administration (5, 10, 20 and 40 mg/kg) produced a dose-dependent reduction in pain threshold of mice as measured by the tail flick technique. This hyperalgesic response of naloxone had a quick onset and was short lived. Pretreatment of animals with morphine (5 mg/kg), clonidine (1 mg/kg) and adenosine (25 mg/kg) antagonized the naloxone-induced hyperalgesia. However, haloperidol and malindone, the dopamine antagonists and cyproheptadine (5 mg/kg), the serotonin antagonist failed to modify the hyperalgesic response of naloxone. Reserpine administration (2.4 mg/kg, ip) also produced hyperalgesia in mice. However reserpine-induced hyperalgesia was accompanied by ptosis, sedation and diarrhoea. Moreover, reserpine-induced hyperalgesia was slow in onset and lasted for around 24 hours unlike naloxone. Further, reserpine-induced hyperalgesia was not modified by clonidine (1 mg/kg) or
adenosine (25 mg/kg). These results indicate that the hyperalgesia evoked by naloxone and reserpine have different characteristics.

Effects of morphine and alpha$_2$ adrenoceptor agonists were studied on cortical EEG pattern in freely moving rats. The bipolar electrodes were implanted chronically in anaesthetized animals and EEG recordings done after recovery from surgical trauma. Clonidine (50, 150, 300 µg/kg) synchronized cortical EEG pattern within 15-75 min of its administration in a dose-dependent manner. Morphine also showed a similar dose related EEG synchrony in rats. Naloxone pretreatment antagonized the synchronizing effect of morphine but did not modify that of clonidine. Yohimbine pretreatment, on the other hand, diminished the effects of both the drugs. The other two alpha$_2$ adrenoceptor agonists, viz. guanfacine and B-HT 920 elicited clonidine-like effects on cortical EEG. The possibility of an opioid-alpha$_2$ receptor interaction mediating EEG changes is discussed. These findings, on analgesia, hyperalgesia and EEG studies collectively highlight an important role for alpha$_2$ adrenoceptors in some of the actions of morphine. It is speculated that the activation of opioid receptor may
trigger in some manner the stimulation of \( \alpha_2 \)-adrenoceptor.

**POTENTIAL OF \( \alpha_2 \) ADRENOCEPTOR AGONISTS IN ALCOHOL ABSTINENCE SYNDROME.**

Various \( \alpha_2 \) adrenoceptor agonists were assessed for their effects on alcohol abstinence syndrome in rats. In the first experimental model, groups of Wistar rats were made alcohol dependent by feeding alcohol together with sweetened milk for 15 days. The volume of fluid intake was measured every 12 h to determine daily ethanol consumption. Abstinence signs following abrupt alcohol withdrawal were observed in control as well as test groups receiving various \( \alpha_2 \) adrenoceptor agonists. Clonidine, guanfacine and B-HT 920, in equimolar concentration (0.5 \( \mu \)M/kg), effectively attenuated the various abstinence signs, which developed after alcohol withdrawal. In the other experimental model, rats were subjected to cold water immersion to induce wet shakes. The inhibitory action of \( \alpha_2 \) adrenoceptor agonists was assessed in this test model. Clonidine, guanfacine and B-HT 920 markedly suppressed the cold water immersion-induced wet shakes and pretreatment with yohimbine (0.1 and 2.0 \( \mu \)M/kg) reversed this
inhibitory effect. The present data reveal the possible therapeutic potential of alpha\textsubscript{2} adrenoceptor agonists in alleviating alcohol abstinence syndrome, and suggest that the resultant reduced noradrenergic activity may be responsible for the beneficial action.

\section*{Alpha\textsubscript{2} Adrenoceptor Mediated Slow and Sustained Contractions of Frog Skeletal Muscle}

Effect of various concentrations of clonidine (1.9x10\textsuperscript{-11} to 1.9x10\textsuperscript{-6}M) was investigated on rectus abdominis muscle of frog. In lower concentrations it neither had any effect \textit{per se} nor did it alter the contractile effect of acetylcholine. However, a higher concentration of clonidine (1.9x10\textsuperscript{-6}M) evoked characteristic slow but sustained contractions. Such an effect was not observed with epinephrine, norepinephrine or isoprenaline. Clonidine-induced sustained contractions were resistant to d-tubocurarine (2.5x10\textsuperscript{-9}M) but susceptible to blockade by yohimbine (5.1x10\textsuperscript{-8}M), an alpha\textsubscript{2} antagonist as well as by verapamil (2.0x10\textsuperscript{-8}M), a calcium channel blocker. Probably activation of post synaptic alpha\textsubscript{2} adrenoceptors by clonidine was responsible for the observed effect. This is further supported by the
observation that guanfacine, a more specific alpha$_2$ adrenoceptor agonist also evoked identical, slow and sustained contractions in frog skeletal muscle, which could be readily reversed by yohimbine.

In conclusion, the ability of clonidine to modify various behavioural functions (such as analgesia, hyperalgesia, despair behaviour, cortical EEG pattern, alcohol abstinence signs etc.) and its potential as an experimental tool was investigated using frogs, mice and the rats. The findings were confirmed using two other more specific alpha$_2$ adrenoceptor agonists viz. B-HT 920 and guanfacine. In the present study clonidine produced a dose-dependent behavioural despair in mice, sensitive to reversal by antidepressants. Clonidine-induced behavioural despair appears to be mediated through its presynaptic action on alpha$_2$ adrenoceptors resulting in reduced central noradrenergic outflow. This phenomenon may serve as a useful test system to screen newer agents with antidepressant potential. This suggestion is further supported by the electroencephalographic data. Clonidine-induced EEG synchronization in conscious rats was reduced by acute treatment and abolished by chronic treatment.
with the antidepressant drugs. Diazepam, an anticonvulsant and a skeletal muscle relaxant did not interfere in the proposed test system. The sensitivity of the test system was not modified by the variation in water temperature from 22°C to 40°C. The involvement of β-adrenergic system in the forced swimming-induced immobility of mice is shown using β-adrenergic agonists and antagonists. The present study highlights an important role for α₂ adrenoceptors in the mechanism of pain transmission. It is speculated that an opioid receptor may be linked to its effector mechanism through an α₂ adrenoceptor. This would account for various clonidine-like effects of morphine and accommodate the fact that yohimbine and not naloxone attenuates the effects of both the drugs. The findings using isolated rectus abdominis muscle indicate the occurrence of dormant α₂ adrenoceptors in the frog skeletal muscle. The present study also highlights possible therapeutic potential of α₂ adrenoceptor agonists in alleviating alcohol abstinence syndrome.