DISCUSSION
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The existence of presynaptic alpha adrenoceptors and their role in the autoinhibition of transmitter release from noradrenaline neurones (Starke, 1977; Langer, 1974; Vizi, 1979) form a relatively new concept, widening our understanding about the mechanisms regulating neurotransmitter release. The functional differences between pre- and postsynaptic alpha adrenoceptors were first observed on rabbit heart and cat spleen preparations. In the rabbit heart, the relative potencies of phenylephrine, oxymetazoline and naphazoline at the presynaptic site did not agree with their relative potencies at the postsynaptic site (Starke, 1972a). In the cat spleen, phenoxybenzamine was more potent in blocking the postsynaptic receptors than the presynaptic ones (Langer, 1974). Clonidine, alpha methylnoradrenaline and tramazoline preferentially activated the presynaptic alpha₂ adrenoceptors, whereas phenylephrine and methoxamine preferentially activated the postsynaptic...
alpha₁ adrenoceptors (Wikberg, 1978; Starke, et al., 1975b; Starke, 1981). Starke et al. (1974) demonstrated that clonidine selectively activated the presynaptic alpha₂ adrenoceptors in the rabbit pulmonary artery. Since then, clonidine has been used not only for the activation of alpha₂ adrenoceptors in functional experiments but also for their labelling in radioligand-binding studies. In spite of some contradictory reports available on the presence of the regulatory alpha₂ adrenoceptors (Kalsner, 1985), the presynaptic receptor concept has now been extended to other putative neurotransmitter systems as well (Mitchell and Martin, 1978; Raiteri et al., 1978; Cerrito and Raiteri, 1979; vizi, 1979). Today several selective alpha₂ adrenoceptor agonists and antagonists are available for experimental as well as clinical use. Yohimbine was perhaps the first agent to be recognised as the antagonist of presynaptic alpha₂ adrenoceptors (Starke et al., 1975a). One of its diastereomers, namely rauwolscine is even more selective. Prazosin, corynanthine, clozapine and azapetine preferentially block the postsynaptic alpha₁ adrenoceptors (Cambridge et al., 1977; Cavero et al., 1977; Doxey et al., 1977). Phenoxybenzamine also primarily blocks postsynaptic alpha
adrenoceptors but, being an irreversible antagonist, is not much in use.

The postsynaptic/presynaptic potency ratio is an essential feature of a drug, deciding how the drug affects the transmission of information through neuroeffector junctions. Alpha_1 adrenoceptor agonists act mainly postsynaptically, where their effect is additive to that of sympathetic nerve stimulation. In contrast, alpha_2 adrenoceptor agonists such as clonidine at low concentrations mainly inhibit the release of noradrenaline and thereby diminish the response to sympathetic nerve stimulation. Furthermore, prazosin-like drugs always inhibit the transmission of information through postsynaptic alpha_1 adrenoceptor blockade. In contrast, low concentrations of alpha_2 adrenoceptor antagonists such as yohimbine primarily interrupt the presynaptic autoinhibition, facilitating the release of noradrenaline and thereby enhancing the transmission of information. This seemingly paradoxical effect of yohimbine was observed long ago, even before the introduction of the presynaptic receptor concept (Bacq, 1935).

In recent years, B-HT 920 and guanfacine have been identified as selective alpha_2 adrenoceptor
agonists (Kobinger and Pichler, 1981; Mottram, 1983; Dausse et al., 1983; Jarrot et al., 1982). These agents, though, structurally unrelated (Fig 1) to phenethylamine or imidazoline type of adrenoceptor agonists evoke clonidine-like effects. Mottram (1983) has shown that B-HT 920, like clonidine and alpha methyl noradrenaline, produces an effective inhibition of the electrically-evoked twitch response in the rat vas deferens and guinea pig ileum. The alpha$_2$ antagonist, yohimbine blocked the inhibitory effects of B-HT 920 on these isolated preparations in a competitive manner (Mottram, 1983). More recently, B-HT 920 and guanfacine have been reported to inhibit phenyl-p-quinone-induced nociceptive response in mice via central alpha$_2$ adrenoceptor activation (Luttinger et al., 1985). The pharmacological results with guanfacine (antihypertensive agent) reveal that the drug decreases the sympathetic tone by activating central alpha adrenoceptors. The antihypertensive effect of guanfacine has been attributed like-clonidine, to its central alpha adrenoceptor stimulating property, resulting in reduced sympathetic tone (Scholtysik, 1980). Biochemical studies have confirmed the above mode of action for guanfacine (Scholtysik et al., 1975;
Scholtysik, 1980). However, clonidine and guanfacine differed from each other in some respects. Clonidine (0.5 mg/kg orally) inhibited the H 44/68 (methylester of alpha methyltyrosine)-induced disappearance of dopamine in the rat corpus striatum by nearly 20% (P < 0.01), whereas guanfacine had no effect up to 10 mg/kg orally (Scholtysik, 1980). Furthermore, stimulation of central histamine -H₂ receptors has been described for clonidine, based on antagonistic effects by metiamide (Karppanen et al., 1976; Finch et al., 1978). On the other hand, the cardiovascular effects of guanfacine were not influenced by metiamide. Another point of difference concerned the central sedative side effect. In conscious dogs, the lowest dose for a detectable sedative action was 3 mg/kg (sc) for guanfacine and 0.03 mg/kg (sc) for clonidine (Scholtysik et al., 1975).

Sedation is a prominent side effect of alpha methyldopa and clonidine-like drugs. The following observations suggested that the sedative effect was mediated by central alpha₂ adrenoceptors. Sleep produced in chicks by clonidine was antagonized by yohimbine, phentolamine, tolazoline and piperoxan but not by azapetine and phenoxybenzamine (Delbarre and Schmitt,
In chicks with incomplete blood-brain barrier the potency of agonists in inducing sleep declined in the order clonidine > alpha methyl-noradrenaline, naphazoline > noradrenaline (Fugner and Hoefke 1971). In rats the sedative potencies of intracerebroventricularly injected agonists declined in the order clonidine > xylazine, naphazoline > methoxamine. Whereas, phenylephrine was inactive. The sedative effect of clonidine was antagonized by intracerebroventricular injection of phentolamine, yohimbine, piperoxan and tolazoline but not by labetalol, thymoxamine or prazosin (Drew et al., 1979). Essentially similar results were obtained in rats by other investigators (Delini-Stula et al., 1979; Nomura et al., 1980). The question again arises as to whether the sedation-mediating sites, if \( \alpha_2 \), are autoreceptors. Since, both sedation and inhibition of noradrenaline release were produced by low doses of clonidine, this does seem possible. In further support of this view, clonidine no longer caused sedation after catecholamine depletion, but, on the contrary, caused locomotor stimulation (Zebrowska-Lupina et al., 1977; Strombom and Svenson, 1980).

These observations suggested a definite role for \( \alpha_2 \)
adrenoceptors in the maintenance of certain behavi-
oural functions. A physiologic dysfunction could 
result from up or down regulation of these central 
alpha₂ adrenoceptors.

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

When forced to swim in a confined space, 
rats or mice after an initial phase of vigorous activity, 
cease to struggle, surrendering themselves to the 
experimental conditions. Porsolt et al. (1978a) 
suggested that this helplessness or despair behaviour 
reflected a state of lowered mood in laboratory animals 
and could serve as a valuable test for screening anti-
depressant drugs. It has recently been shown (Kulkarni 
and Mehta, 1985) that purine nucleosides augment this 
helplessness probably through their presynaptic 
inhibitory action on noradrenergic nerve terminals 
(Fig 21). In the present study, clonidine, an alpha₂ 
adrenoceptor agonist, induced a dose-dependent behavi-
oural despaire in mice as judged by the significant 
enhancement in the immobility duration. This 
characteristic effect of clonidine (Fig 3) was reversed 
by acute as well as chronic treatment with tricyclic 
antidepressants (viz. imipramine, desipramine, trimi-
**FIG 21.** Inhibition of norepinephrine release by purine nucleosides leading to depression and its reversal by pretreatment with various anti-depressants and psychostimulant
pramine, amitriptyline, nortriptyline, doxepin). Tranylcypromine (MAO inhibitor) and amphetamine (psychostimulant) also reversed clonidine-induced behavioural despair. The reversal of clonidine-induced behavioural despair by these drugs may be a consequence of enhanced noradrenergic activity resulting from inhibition of norepinephrine uptake, increased norepinephrine release or MAO inhibition. Furthermore, antidepressants administered chronically, may be enhancing norepinephrine function by bringing about down regulation of presynaptic alpha₂ adrenoceptors. This suggestion is in line with the studies of investigators, who, have shown reduction in alpha₂ adrenoceptor sensitivity using ligand binding (Cohen et al., 1982); peripheral tissue (Finberg and Tal, 1985); electrophysiological (Svensson and Usdin, 1978) and biochemical (Sugrue, 1980) techniques.

The reversal of clonidine-induced enhanced immobility could be easily observed and may be made use of to study the antidepressant potential of newer agents. The test procedure is simple enough to lend itself to minor experimental manipulations. The sensitivity of the proposed test system was not modified by the variation in water-temperature from 22°C to 40°C.
Moreover, diazepam, a skeletal muscle relaxant did not interfere in the test system, thereby supporting its validity.

The sedative as well as the suppressant effect of clonidine on the locomotor activity is likely to overlap the behavioural depressive signs. The present study indicates that the despair behaviour evoked by clonidine may be dissociable from its sedative effect and is sensitive to antidepressant treatment. It may be worthwhile to note that the animals treated with a sedative dose of pentobarbitone showed a sinking tendency when forced to swim in water, unlike clonidine.

Biochemical and neurophysiological studies indicate that the depressive state produced by clonidine is associated with reduced noradrenergic activity. Tang et al. (1979) and Warsh et al. (1981) found decreased brain 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in rats treated with clonidine suggesting reduced turnover of norepinephrine. Furthermore, the rate of firing of ascending noradrenergic neurons in the brain was reduced by both, intravenous injection and direct application of clonidine in the vicinity of the locus coeruleus pericarya (Svensson et al., 1975).
In the present study, an ineffective concentration of adenosine potentiated the submaximal effect of clonidine but failed to modify its peak response indicating that the two agents are probably acting at the presynaptic site, regulating norepinephrine outflow. Since yohimbine, which preferentially blocks presynaptic alpha adrenoceptors (Starke et al., 1975a) antagonised this action of clonidine, an important role for presynaptic alpha adrenoceptors in the pathogenesis of depression is speculated. Furthermore, guanfacine (Dausse et al., 1983) and B-HT 920 (Mottram, 1983), the more specific alpha₂ adrenoceptor agonists also exerted clonidine-like effects.

The present findings propose a simple, sensitive and reliable test model to induce depression-like syndrome in laboratory animals and underline noradrenergic involvement in the pathogenesis of depression.

BETA ADRENOCEPTOR INVOLVEMENT IN FORCED SWIMMING-INDUCED IMMOBILITY OF MICE

Mice, when exposed to a mild aversive situation from which there is no possibility of an escape, eventually cease struggling and assume a typical
immobile posture (Porsolt, et al., 1978a; Kulkarni and Mehta, 1985). Low doses (10 μg-2 mg/kg) of isoprenaline did not modify the immobility duration of mice. Therefore higher doses were employed to assess β-adrenoceptor involvement in forced swimming-induced despair behaviour. Isoprenaline, in high doses prolonged the behavioural despair of mice during the six min test. Propranolol administration antagonized the effect of isoprenaline, suggesting a role for β-adrenoceptors in the behavioural despair. Since atenolol and metoprolol, both (β₁-antagonists) antagonized the effect of isoprenaline, β₁-subtype of adrenoceptors appear to be primarily involved in the prolongation of despair behaviour of mice. Our observations are in line with the findings of Kitada et al. (1983) who have shown that the immobility-reducing action of antidepressants is modified by icv administration of β-adrenoceptor agonists and antagonists. Isoprenaline (a β-agonist) diminished while atenolol and practolol, (the β₁-antagonists) but not IPS-339, (a β₂-antagonist) potentiated the action of desipramine (Kitada, et al., 1983).

In the present study, acute as well as chronic treatment with imipramine protected the animals
from isoprenaline-induced prolongation of immobility duration. This observation indicates the possibility of an involvement of β-adrenergic mechanism in the therapeutic action of imipramine-like drugs. This suggestion is in agreement with the studies of Bergstrom and Kellar (1979) who have shown that several tricyclic antidepressants on chronic treatment reduce β-adrenoeceptor density selectively. In this regard, it is noteworthy that a reduction in β-adrenoeceptor density and a desensitization of noradrenergic c-AMP generating systems in the brains of rats was induced by chronic administration of antidepressants (Vetulani et al., 1976; Wolfe et al., 1978).

In the present investigation clonidine, which exerts an inhibitory influence on norepinephrine release; adenosine, which probably reduces norepinephrine outflow through their action on presynaptic purinoceptors and reserpine which depletes norepinephrine stores, produced profound behavioural despair in mice (Kulkarni and Mehta, 1985) analogous to that observed with isoprenaline. It is therefore speculated that isoprenaline may be reducing the availability of norepinephrine to its effector site in some manner. This speculation is supported by the studies of following
investigators. Maggie et al. (1980) have demonstrated in rat brain slices that isoprenaline induces an increase in alpha$_2$ adrenoceptors, which mediate the negative feedback of norepinephrine release. Isoprenaline stimulated the synthesis of PGE$_2$ in rat brain slices, which has been shown to suppress the evoked release of norepinephrine in vitro (Bergstrom et al., 1973; Hillier and Templeton, 1980; Taube et al., 1977). Furthermore, propranolol ($\beta$-antagonist) has been reported to increase norepinephrine metabolites in several rat brain regions (Fludder and Leonard, 1979). Also, it may be noteworthy that central $\beta_1$-adrenergic mechanisms inhibit the action of desipramine and of presynaptic noradrenergic neurons in the rat brains (Miyauchi et al., 1984). However, the possibility that the immobility-enhancing effect of isoprenaline may be a consequence of its cardiovascular actions can not be ruled out. Particularly, since isoprenaline (an energy wasting agent)-induced immobility may represent an energy-saving strategy of mice.

Salbutamol, a $\beta_2$-adrenoceptor agonist reduced the immobility duration of mice in the present study. This observation is in line with the studies of Widlocher et al. (1977) who have shown that salbutamol
produces powerful antidepressant effect in man. Further, the immobility-reducing action of desipramine in forced swimming rats was attenuated by intracerebroventricular injection of isoprenaline and potentiated by atenolol (icv), a $\beta_1$-adrenoceptor antagonist (Miyauchi et al., 1984). These findings suggest that central $\beta_1$- and $\beta_2$- subtype of adrenoceptors may be acting in opposite directions to modify the behavioural despair. 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

Tricyclic antidepressant drugs such as desipramine and amitriptyline inhibit the neuronal uptake of noradrenaline and consequently elevate synaptic levels of the neurotransmitter. This mechanism of action has been implicated in the antidepressant action of tricyclic antidepressant drugs. There is however, a temporal dissociation between the blockade of neuronal uptake, which occurs within hours of commencing therapy and the onset of antidepressant effect, which may take 2-3 weeks to become evident. Crews and Smith (1978) have shown that prejunctional
alpha_2 adrenoceptors become subsensitive after chronic treatment with desipramine, thus allowing a marked accumulation of noradrenaline within the synapse. The slow development of alpha_2 adrenoceptor subsensitivity on chronic treatment may explain the delayed onset of the therapeutic effect of tricyclic antidepressant drugs. Consistent with this hypothesis, Svensson and Usdin (1978) demonstrated reduced alpha_2 adrenoceptor sensitivity in the locus coeruleus of rats treated chronically with imipramine. Furthermore, prolonged treatment of rats with desipramine resulted in an increase in both noradrenaline turnover and MOPEG levels in the brain, whereas acute administration reduced both noradrenaline turnover and MOPEG levels (Sugrue, 1980).

In the present study, clonidine, in micromolar doses, synchronized cortical EEG pattern of rats in a dose-dependent manner. This effect of clonidine was antagonized by yohimbine pretreatment thereby suggesting that it is an alpha_2 adrenoceptor mediated effect. This finding is in line with that of Florio et al (1975). Further, our observation that chronic treatment with imipramine antagonized the EEG synchronizing effect of clonidine is consistent
with the studies of Passarelli and Scotti de Carolis (1983).

Clonidine evoked signs of behavioural depression in rats in the present study. This finding confirms the work of other investigators, who have shown that clonidine induces behavioural depression uniformly in rats, mice and the Mongolian gerbil (Drew et al., 1979; Dwoskin and Sparber, 1983; Gower and Marriott, 1980; Kostowski and Malatynska, 1983; VanderLaan et al., 1985). Recent studies have shown that chronic administration of antidepressants produce compensatory changes in noradrenergic neurotransmission (Finberg and Tal, 1985; Kostowski and Malatynska, 1983; Spyraki and Fibiger, 1980). Spyraki and Fibiger (1980) have reported that 15-day but not 2-day pretreatment with desipramine significantly reduce the effect of clonidine on exploratory behaviour. Whereas, Kostowski and Malatynska (1983) reported that a 5-day treatment with antidepressants was sufficient to antagonize the depressant effect of clonidine on locomotor and exploratory activity. Our results indicate that chronic treatment of 8 days with tricyclic antidepressants was essential to produce compensatory changes in noradrenergic neurotransmission.
counteracting the clonidine effect.

Acute as well as chronic treatment with tricyclic antidepressants inhibited clonidine-induced EEG synchrony. This inhibitory effect of tricyclics may be a consequence of enhanced noradrenergic activity resulting from either inhibition of norepinephrine uptake and/or anticoncidine effect via interaction with presynaptic alpha2 adrenoceptors. This speculation is in parallel with the recent studies of Pinberg and Tal (1985); showing down-regulation of presynaptic alpha2 adrenoceptors by chronic treatment with imipramine-like drugs. Furthermore, two other more specific alpha2 adrenoceptor agonists viz. guanfacine and B-HT 920 synchronized cortical EEG pattern of rats like clonidine.

In conclusion (Fig 22) we suggest that the antagonism of clonidine induced EEG synchronization in conscious animals could serve as a valuable test for screening of agents with antidepressant potential. This suggestion is in line with the findings discussed under the subheading, Clonidine-induced behavioural despair: An experimental model to detect the antidepressant activity.
CLONIDINE
B-HT 920
GUANFACINE

YOHIMBINE

EEG SYNCHRONY
DESPAIR BEHAVIOR

ANTIDEPRESSANTS (ACUTE/CHRONIC)

FIG. 22 USEFUL AND SENSITIVE TEST SYSTEM TO SCREEN ANTIDEPRESSANTS.
ROLE OF ALPHA₂ ADRENOCEPTORS IN ANTINOCICEPTION

Electrical stimulation of brain stem noradrenergic nuclei is reported to produce analgesia (Segal and Sandberg, 1977; Akaike et al., 1978) and iontophoretically applied norepinephrine inhibits the discharge of dorsal horn neurons evoked by noxious stimuli (Belcher et al., 1978; Headley et al., 1978). Both these findings suggested an intimate association of alpha adrenoceptors with antinociception. These alpha adrenoceptors have been lately classified into subtypes as alpha₁ and alpha₂ (Langer, 1974; Berthelsen and Pettinger, 1977). Furthermore, not all noradrenergic blocking drugs affect the activity of clonidine (Anden et al., 1976; Fielding et al., 1978). Also clonidine has been reported to stimulate central epinephrine receptors (Bolme et al., 1974), histamine-H₂ receptors (Sastry and Phillis, 1977) and to inhibit the activity of serotonergic neurones (Maj et al., 1973, Svensson et al., 1975). Therefore, in the present investigation, an attempt has been made to estimate a statistical constant to study the antinociceptive phenomenon.

\( pA_2 \) is defined as the -ve log of the molar concentration of the antagonist that demands doubling of the dose of the agonist to compensate for its action.
These values reflect the affinity constant of an antagonist for the receptor. Identical $pA_2$ values in various test preparations suggest the involvement of similar receptor types. Based on this principle, Arunlakshana and Schild (1959) classified cholinergic and histaminergic receptors in various tissue preparations, viz. guinea-pig ileum, guinea-pig tracheal chain, rat fundus, frog rectus and guinea-pig perfused lung. Smits and Takemori (1970) extending this $pA$ concept to in vivo studies characterized the analgesic receptors, employing narcotic agonists and antagonists. Apparent $pA_2$ values for the clonidine-yohimbine pair and for the morphine-naloxone pair, in the present investigation were estimated to be 7.45 and 7.09, respectively. These values are in close agreement with the report of Hayashi and Takemori (1971) who, employing three different analgesic assays (viz. tail flick, hot plate and writhing technique) demonstrated that $pA_2$ values for morphine-naloxone pair were identical, irrespective of the test procedure used. Recently, a similar $pA_2$ value is reported for gossypin-naloxone (7.38) employing the acetic acid-induced writhing technique in mice (Viswanathan et al., 1984). These results indicated that a common antinociceptive site or
some common pathway may be involved in all these studies, as would be expected from close $\mathrm{pA_2}$ values.

Since both, clonidine, an $\alpha_2$ agonist, and morphine, an opioid, produced profound antinociception in the tail flick test which was sensitive to yohimbine blockade, a common antinociceptive mechanism in the action of these agonists could be speculated. It has been shown earlier that acute environmental heat and immobilization-induced analgesia in rats and mice is a catecholamine-mediated response, as it was abolished by pretreatment with both catecholamine depleters and adrenoceptor blockers (Kulkarni, 1980a). It is therefore speculated that an opioid receptor may be connected to its effector mechanism through an $\alpha_2$ adrenoceptor.

Several chemically and pharmacologically unrelated substances (such as opioids, clonidine, B-HT 920, oxymetazoline, gossypin) have been shown to produce analgesia in laboratory animals, using various test procedures. On the other hand, only a few substances are reported to evoke hyperalgesia in laboratory animals. Such substances can give important clues to elucidate the mode of pain transmission. Naloxone administration produced a dose-dependent reduction in
pain threshold of mice in the present study. This hyperalgesic response of naloxone had a quick onset and the effect was short lived. Pretreatment of mice with morphine, adenosine and clonidine antagonized the naloxone-induced hyperalgesia. In contrast, dopamine and serotonin antagonists failed to modify the response of naloxone. Reserpine treatment also produced hyperalgesia in mice. However, reserpine-induced hyperalgesia differed from naloxone-induced hyperalgesia in some respects. Reserpine-induced hyperalgesia was accompanied by ptosis, sedation and diarrhoea. Further, reserpine-induced hyperalgesia had a delayed onset and the effect lasted for around 24h, unlike naloxone. Recently, Kulkarni and Robert (1982) showed, using several serotonergic agents that reserpine-induced hyperalgesia was serotonin-dependent.

Endogenous opioids are now known to regulate pain perception and transmission. The fact that naloxone, an opioid antagonist produced morphine-sensitive hyperalgesia in the present study, further substantiates this contention. Clonidine reversed the hyperalgesic response of naloxone but the converse was not true. These findings extend support to the speculation that an opioid receptor may be linked to its
effector mechanism through an alpha adrenergic receptor. The fact that adenosine, like-clonidine may be exerting an inhibitory influence on noradrenaline release (Kulkarni and Mehta, 1985) explains the antagonism of naloxone-induced hyperalgesia by adenosine pretreatment.

Clonidine is a potent alpha adrenoceptor stimulating agent, acting on both alpha_1 and alpha_2 adrenoceptors. In low doses, clonidine decreases noradrenergic neurotransmission through activation of presynaptic alpha_2 adrenoceptors whereas, in high doses it activates postsynaptic alpha_1 adrenoceptors (Anden et al., 1970b; Starke, 1977). In the present investigation clonidine, in micromolar doses synchronized cortical EEG pattern of rats in a dose-dependent manner. Morphine, an opioid, also produced dose-dependent EEG synchrony in rats. These results are consistent with the report of Florio et al., (1975).

Acute administration of morphine and clonidine have been shown to have similar pharmacological profiles in many respects although, the two agents stimulate independent population of receptors viz. opioid and alpha receptors, respectively. For instance, both the drugs produce profound antinociception in laboratory animals (Fielding et al., 1978; Luttinger
et al., 1985), as well as in human beings (Gordh and Tamsen, 1983). They lower body temperature (Cowan and MacFarland, 1976; Kulkarni, 1980b) blood pressure (Schmitt et al., 1979) and heart rate (De Jonge et al., 1981; Laubie et al., 1979) of laboratory animals. These common pharmacological properties of morphine and clonidine indicate the possibility of an inter-link between alpha_2 adrenoceptors and opioid receptors.

Opiates, enkephalins and clonidine, all depress spontaneous firing of noradrenergic neurons in locus coeruleus and inhibit depolarization-induced release of norepinephrine from cerebral slices (Bird and Kuhar, 1977; Montel et al., 1975; Svensson et al., 1975). These findings highlight the involvement of noradrenergic system in the actions of morphine (Fig 23). The possibility of release of endogenous opioids by clonidine, accounting for its morphine-like effects, is ruled out by the fact that naloxone (an opioid antagonist) failed to block both, its EEG synchronizing effect as well as analgesia.

In the present study, EEG synchronization produced by both, clonidine and morphine was antagonized by low doses of yohimbine, which preferentially blocks alpha_2 adrenoceptors. Furthermore, two other
FIG 23. Inhibition of norepinephrine release by enkephalins and clonidine from the locus coeruleus neuron.
alpha₂ agonists, viz. guanfacine and B-HT 920 elicited morphine-like synchronized EEG patterns in rats. These findings suggest an involvement of alpha₂ adrenoceptors in some of the actions of morphine. It is noteworthy that several alpha₂ agonists (viz. clonidine, guanfacine, B-HT 920, lofexidine, guanabenz, ICI-106270, xylazine etc.) have been shown to produce appreciable antinociceptive effect (Luttinger et al., 1985), analogous to morphine.

The present data suggests an interlink between opioid receptors and alpha₂ adrenoceptors. It is speculated that activation of opioid receptors trigger in some way the stimulation of alpha₂ adrenoceptors, resulting in reduced noradrenergic outflow. This would account for various clonidine-like effects of morphine and accommodate the fact that yohimbine, an alpha₂ adrenoceptor antagonist attenuates the effects of both the drugs.

POTENTIAL OF ALPHA₂ ADRENOCEPTOR AGONISTS IN ALCOHOL ABSTINENCE SYNDROME

An abstinence syndrome may be regarded as an expression of the body's reaction to no longer receiving an addictive agent. In the present investigation, various abstinence signs, such as body tremors, escape attempts, jumping, teeth chattering, increased locomotor activity,
blanching of ears and wet dog shakes, were markedly attenuated by all three alpha2 adrenoceptor agonists employed, thereby revealing the possible therapeutic potential of this class of drugs. Clinical studies with clonidine (Bjorkqvist 1975; Gold et al., 1978; Wilkins et al. 1983) further substantiate this speculation. The remarkable reduction in fluid intake by control animals on alcohol withdrawal possibly reflected their reluctance to drink milk which no longer contained ethanol.

Increased locomotor activity may reflect the state of anxiety prevailing on abrupt alcohol withdrawal. Peripheral administration of clonidine has been reported to cause a dose-dependent decrease in locomotor activity (Maj et al., 1972, 1975) and to antagonize ethanol-induced motor stimulation (Strombom et al., 1977). Cholinergic (Maj et al., 1975), dopaminergic (Maj et al., 1972) and noradrenergic (Anden et al., 1976) mechanisms have all been implicated in the above action of clonidine. In the present investigation, not only clonidine but also two other more selective alpha2 adrenoceptor agonists, viz. B-HT 920 and guanfacine, supressed ethanol withdrawal-induced motor stimulation, extending support to noradrenergic involvement.
Furthermore, some evidence has accumulated indicating that manifestations of the abstinence syndrome are mediated by hyperactivity of catecholamines in man (Freedman et al., 1982). More recently, body shakes have been suggested to be more sensitive to $\alpha_2$ adrenoceptor agonists than changes in locomotor activity; thus the antiwithdrawal actions of these drugs might be intrinsically higher than their sedative action (Van Der Laan et al., 1985). The results obtained in the present study using the cold water immersion technique confirm this contention.

Head and body shakes constitute normal behavioral patterns in rats (Silverman 1965) and develop as a consequence of internal or external stimulation. Wei et al. (1973) have shown, with the aid of brain lesion studies, that cold water-induced wet shakes share common neural pathways with wet dog shakes (Martin et al. 1963) observed in the abstinence syndrome. Since all the $\alpha_2$ adrenoceptor agonists employed reduced the frequency of wet shakes and yohimbine, an $\alpha_2$ adrenoceptor antagonist (Starke et al., 1975a), reversed this action, wet shake behaviour seems to be reciprocally related to $\alpha_2$ adrenoceptor activation and vice versa. The beneficial action of clonidine, B-HT 920
and guanfacine may be mediated through activation of presynaptic alpha\textsubscript{2} adrenoceptors resulting in decreased central noradrenergic activity. This suggestion is in line with the recent studies (Franz et al., 1982; Wilkins et al., 1983) and is consistent with the clinical observation that norepinephrine turnover is increased more in patients with psychiatric symptoms than in those without symptoms (Borg et al. 1983).

**ALPHA\textsubscript{2} ADRENOCEPTOR MEDIATED SLOW AND SUSTAINED CONTRACTIONS OF FROG SKELETAL MUSCLE**

Recently it has been suggested that the classification of alpha adrenoceptors into subtypes be according to pharmacological effects rather than based on their pre- or post-synaptic location (Wikberg, 1978). Receptors more sensitive to clonidine than to phenylephrine and more susceptible to blockade by yohimbine than by prazosin have been designated as alpha\textsubscript{2} and receptors at which the effectiveness of these drugs is in reverse order have been designated as alpha\textsubscript{1}. Docherty et al. (1979) and Timmermans and Van Zwieten (1980) have reported that rat vasculature has both alpha\textsubscript{1} and alpha\textsubscript{2} receptors as mediators of pressor response. Likewise Constantine et al. (1980) have demonstrated the presence of both types of alpha adrenoceptors in dog vasculature.
The present study suggests the occurrence of alpha$_2$ adrenoceptors in frog skeletal muscle.

In the present study both clonidine and guanfacine evoked contractions in frog rectus abdominis muscle, which exceeded the acetylcholine-induced peak. This response of alpha$_2$ adrenoceptor agonist was resistant to d-tubocurarine blockade. Moreover, the nature of these contractions differed from those due to acetylcholine in that they were slow and sustained, suggesting the involvement of a separate receptor site. Since pretreatment with yohimbine, which preferentially blocks alpha$_2$ adrenoceptors (Starke et al., 1975a), antagonized clonidine and guanfacine-induced typical contractions, it suggested the presence of receptors akin to alpha$_2$ adrenoceptors in frog rectus abdominis muscle. These receptors appear to be dormant in nature as high concentrations of alpha$_2$ agonists were needed to elicit a response. The fact that epinephrine, norepinephrine and isoprenaline did not evoke any contractions, implies either poor affinity or inability of these agents to activate these dormant receptors. Moreover, this action of clonidine appears to be unrelated to its anticonvulsant effect (Kulkarni, 1981) observed in rats at low doses.
Van Meel et al. (1981) suggested that $\alpha_1$-stimulated contractions are mediated by the release of intracellularly stored calcium, while $\alpha_2$-stimulated contractions are dependent on extracellular calcium. In the present investigation, the blockade of clonidine-induced sustained contractions by verapamil, a calcium channel blocking agent, further supports the presence of $\alpha_2$ adrenoceptors in frog rectus abdominis muscle which upon activation evoke sustained contractions probably through increased influx of extracellular calcium.