SUMMARY AND CONCLUSION

STUDIES ON PURINOCEPTORS IN THE RAT ANOCOCYGEUS MUSCLE PREPARATION: MODULATORY ROLE OF GABA_\textsubscript{B}-ERGIC AGENTS

Electrically evoked frequency-dependent contractions of the rat anococcygeus muscle well inhibited by adenosinergic agents, adenosine, 2-chloroadenosine and N^6-cyclohexyladenosine. Adenosine and N^6-cyclohexyladenosine did not alter the responses of exogenously applied norepinephrine. N^6-cyclohexyladenosine-induced inhibition was found to be theophylline sensitive. The inhibitory effect of adenosinergic agents appear to be mediated via presynaptic A_1-adenosine receptors. ATP and \(\beta,\gamma\)-methylene ATP produced concentration dependent contractions of the anococcygeus muscle. \(\beta,\gamma\)-methylene ATP produced more potent excitatory response than ATP. Repeated administration of ATP and \(\beta,\gamma\)-methylene ATP resulted in blockade of the responses to ATP and \(\beta,\gamma\)-methylene ATP. ATP at the concentration which had no effect on the basal tension of the muscle potentiated the field stimulation responses. ATP-induced contractions were inhibited by GABA, (±)baclofen and (-)baclofen.
respectively. (-) Baclofen produced more pronounced inhibition of ATP-induced contractions as compared to GABA and (+) baclofen. (+) Baclofen and (-) baclofen also inhibited the electrically evoked contractions of the anococcygeus muscle. The results indicate the presence of presynaptic A₁ adenosine receptors, excitatory P₂x purinoceptors in the anococcygeus muscle and GABA₂-ergic agents modulate the excitatory ATP-responses of the anococcygeus muscle.

INVOLVEMENT OF POSTSYNAPTIC ADRENERGIC MECHANISM(S) IN THE ATP-CONTRACTIONS OF THE RAT ANOCOCYGEUS MUSCLE AND THE EFFECT OF EXTRACELLULAR Ca²⁺

Clonidine, ICI-106 270, B-HT-920 and guanfacine, agonists at α₂-adrenoceptors, produced concentration-dependent contractions of the rat anococcygeus muscle. The order of potency of these agonists in producing the response was clonidine ICI-106 270 guanfacine B-HT 920. Yohimbine (10⁻⁶M) competitively antagonized the contractile response due to these agents acting at α₂-adrenoceptors.

ATP (10⁻⁶M - 10⁻⁴M) also produced concentration-dependent contractions of the rat anococcygeus muscle. Prazosin (10⁻⁸M) an α₁-blocker, significantly antagonized ATP-induced contractions in a competitive manner. ATP (10⁻⁷M) in a concentration that had no effect on basal tension of
the preparation, tends to potentiate phenylephrine responses, though not significantly. The sensitivity of the anococcygeus muscle to ATP was enhanced after 24 hr (maximum catecholamine depletion period) of reserpine (5 mg/kg, IP) treatment. Yohimbine \(10^{-6}\) M, a selective \(\alpha_2\)-blocker, significantly antagonized ATP-induced contractions and shifted the ATP concentration-response curve towards right. The antagonism was found to be competitive. The ATP-contractions were also sensitive to inhibition by reduced calcium contents (quarter or calcium free) in the Krebs bicarbonate solution. However, it was observed that ATP was capable of producing contractions of anococcygeus muscle even in Ca-free Krebs bicarbonate solution. These results indicate the presence of postsynaptic \(\alpha_2\)-adrenoceptors in the rat anococcygeus muscle and the ATP-induced contractions may be mediated by both postsynaptic \(\alpha_1\) - and \(\alpha_2\)-adrenoceptors. The possible mechanism of ATP-contraction in calcium deprived anococcygeus muscle is explained.

THE NEUROPROTECTIVE EFFECTS OF ADENOSINERGIC AGENTS, Ro 5-4864 AND CARBAMAZEPINE AGAINST HYPOXIC-NEUROTOXICITY IN MICE

Mice subjected to hypoxic stress resulted in increased respiratory rate, tremor and convulsions followed by death. The latencies for convulsion and death following hypoxic stress were 33.29 ± 1.20 and 34.36 ± 1.16 min, respectively. In the
present study effects of adenosinergic agents, Ro 5-4864, a "peripheral-type" benzodiazepine receptor agonist, and carbamazepine were studied on hypoxic stress-induced neurotoxicity. Adenosinergic agents such as adenosine, 2-chloroadenosine, N6-cyclohexyladenosine and dipyridamole increased the latency for convulsions and death due to hypoxia. Theophylline (50 mg/kg, IP) an adenosine receptor antagonist, reversed this protective effect of adenosinergic agents. Pretreatment with Ro 5-4864 (10, 20 mg/kg, IP) also offered theophylline (50 mg/kg, IP)-sensitive protection against hypoxic stress. Similarly, carbamazepine treatment (10-30 mg/kg, IP) significantly prolonged the latencies for convulsion and death following hypoxic stress. Prior treatment with theophylline (50 mg/kg, IP) reversed the protective effect of carbamazepine, indicating the possible involvement of adenosinergic mechanism in the observed protective effect of carbamazepine. These results indicate that the adenosinergic mechanism may be responsible for the observed neuroprotective effect of these agents against hypoxia.

EFFECT OF MK-801 AND ITS INTERACTION WITH ADENOSINERGIC AGENTS AND CARBAMAZEPINE AGAINST HYPOXIC STRESS-INDUCED NEUROTOXICITY IN MICE

Effects of MK-801, adenosinergic agents and carbamazepine were investigated against hypoxic stress-induced neurotoxicity such as convulsion and death in mice. MK-801,
a N-methyl D-aspartate antagonist, offered significant protective effect against neurotoxicity due to hypoxia. Adenosinergic agents such as adenosine, 2-chloroadenosine, N\textsuperscript{6}-cyclohexyladenosine and dipyridamole produced theophylline sensitive protective effect against hypoxic neurotoxicity as they dose-dependently prolonged the latencies for onset of convulsion and death. Pretreatment with subprotective doses of adenosinergic agents and MK-801 markedly enhanced the neuroprotective effect of the rigid analogues of adenosine namely, 2-chloroadenosine and N\textsuperscript{6}-cyclohexyladenosine. Prior treatment with theophylline reversed this enhanced protective effect. Carbamazepine also exhibited dose-dependent neuroprotective effect and the combined treatment with carbamazepine and MK-801 significantly enhanced the protective effect. These results indicate the possible involvement of adenosinergic mechanisms in preventing the hypoxic stress-induced neurotoxicity and the role of NMDA receptors in hypoxia as MK-801 not only produced effect per se but also potentiated the response due to adenosinergic agents.

ETHANOL AND MK-801 ANTAGONISE CAFFEINE-INDUCED CONVULSIONS IN MICE: ADENOSINERGIC MEDIATION

Effects of ethanol and MK-801 were investigated against caffeine-induced convulsions in mice. Ethanol (1 g/kg, IP) offered significant protection against caffeine-induced convulsions and mortality. Ro 15-4513, an ethanol
antagonist reversed the protective effect of ethanol against caffeine-induced convulsions and mortality. Pretreatment of animals with adenosine or dipyridamole, an adenosine uptake inhibitor significantly prolonged the latencies for myoclonic jerks and convulsions in ethanol treated group against caffeine treatment. Pretreatment of animals with MK-801 also offered significant neuroprotection against caffeine-induced convulsions and mortality. The anticonvulsant effect of MK-801 was not however, potentiated by ethanol. Prior, adenosine treatment significantly potentiated the protective effect of ethanol on mortality due to caffeine treatment. The present observations demonstrate that ethanol and MK-801 are effective against caffeine-induced convulsions and mortality in mice and suggest that adenosinergic system may possibly play a role in the anticonvulsant effects of these agents.

ON THE ANTICONVULSANT ACTIVITY OF CARBAMAZEPINE IN MICE

The interaction of carbamazepine with purinergic system and γ-aminobutyric acid (GABA)-benzodiazepine (BZ)-receptor complex using various convulsants was investigated in mice. CBZ failed to modify caffeine and theophylline-induced convulsions. Picrotoxin and bicuculline-induced convulsions were inhibited by pretreatment of animals with
carbamazepine. Carbamazepine was also effective in inhibiting Ro 5-4864-induced convulsions. It also antagonised the convulsive response due to combined treatment with subconvulsive doses of Ro 5-4864 and theophylline. These results suggest that anticonvulsant effect of carbamazepine may be mediated preferentially via GABA-benzodiazepine-receptor site rather than at the purinoceptors.

BENZODIAZEPINE INVERSE AGONIST, DMCM- AND PERIPHERAL AGONIST, Ro5-4864-INDUCED CONVULSIONS IN MICE: EFFECT OF ADENOSINERGIC AGENTS

Adenosinergic agents such as adenosine, 2-chloroadenosine, N\(^6\)-cyclohexyladenosine produced dose-dependent protective effect against DMCM- and Ro 5-4864-induced convulsions and mortality. N\(^6\)-cyclohexyladenosine produced most significant effect against Ro 5-4864-induced convulsions whereas 2-chloroadenosine was more effective than N\(^6\)-cyclohexyladenosine in antagonising DMCM-induced convulsions. Pretreatment of animals with subprotective doses of adenosine and dipyridamole significantly prolonged the latencies for the onset of myoclonic jerks and convulsions due to both DMCM and Ro 5-4864. DMCM and Ro 5-4864-induced mortality rate was also significantly reduced by pretreatment with subprotective doses of adenosine and dipyridamole. Similarly, subprotective doses of adenosine and
diazepam further delayed the latencies for myoclonic jerks and convulsions due to DMCM and Ro 5-4864 treatment. The results suggest that adenosine and adenosine receptor agonists, 2-chloroadenosine and N⁶-cyclohexyladenosine are protective against both DMCM- and Ro 5-4864 induced convulsions. It is suggested that adenosinergic agents via activation of central A₁ adenosine receptors may modulate the convulsant effects mediated by DMCM and Ro 5-4864. This study further supports the notion that adenosinergic mechanisms mediate neuroprotective effects in the central nervous system.