THE EFFECT OF ACUTE LITHIUM AND AMI-193, A NEW $5HT_2$ ANTAGONIST, ON APOMORPHINE-INDUCED PECKING IN PIGEON

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Abstract- ***Intramascular (IM) administration of apomorphine (a mixed D_1/D_2 dopamine receptors agonist ,0.2-1.6 mg/kg) induced pecking,a stereotype behavior in pigeons in a dose- dependent manner. In this study the effect of lithium (Li^+, 240 mg/kg, IM) and AMI-193(a new 5-HT_2 antagonist, 0.003 mg/pigeon) on apomor-phine-induced pecking (AIP) were investigated. This study showed that Li^+ and AMI-193 did not induce pecking by itself but administration of each of these agents before apomorphine increased and decreased the AIP (apomor-phine 0.8 mg/kg) respectively whereas concomitant use of Li^+(240 mg/kg IM) and AMI-193 decreased AIP significantly.These results suggested that 5-HT_2 antagonists inhibit the inhibitory effect of serotonin on the dopamine release in the raphe-striatal pathway but Li^+can modulate dopamine and serotonin function by different mechanisms and decrease this effect. As a result, it is concluded serotonin can decrease the AIP through 5-HT_2 receptors indirectly by decrease the dopamine release.***

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Key Words: Pecking, serotonin receptors, apomor-phine

INTRODUCTION

Biochemical and pharmacological evidence indicates the existence of three different serotonin (5-HT) receptor families termed $5HT_1$ ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_{1D}$) relating to G protein (1), $5HT_2$ ($5HT_{2A}$, $5HT_{2B}$, $5HT_{2C}$, $5HT_4$) relating to phosphoinositol and $5HT_3$ relating to ion channels (2-4). 5-HT receptors subtypes have been shown to be present in the basal ganglia (5). High to moderate levels of 5-HT_{2A} and 5-HT_{2C} receptor binding sites and the corresponding mRNA are present in several forebrain areas including the basal ganglia and the limbic system.Thus ,high levels of 5-HT_{2A} and 5-HT_{2C} binding sites are found in the caudate nucleus, nucleus accumbens, olfactory tubercle and pyriform cortex (5). Although there is a good correlation between the distribution of 5-HT_{2A} and 5-HT_{2C} binding sites and respective mRNAs (6), the relative distribution of mRNA for 5-HT_{2A} and 5-HT_{2C} receptors differs. Moderate levels of mRNA for both 5-HT_{2A} and 5-HT_{2C} receptors have been detected in substantia nigra (SN), whereas the ventral tegmental area (VTA) contains mRNA for 5-HT_{2C} but not 5-HT_{2A} receptors (6-8). A large scientific literature exits concerning the role of different 5-HT receptor subtypes in the control of brain dopamine-mediated transmission, for example substantial evidence suggests that the functional status of the mesocorticolimbic dopamine (DA) system originating in the VTA is under a phasic and tonic inhibitory control by the 5-HT system that acts by stimulating 5-HT_{2C} receptor subtypes (5).

Lithium (Li^+) is the most specific drug used for the treatment and prevention of recurrent manic-depressive disorders (14-16).The molecular mechanisms related to the therapeutic action of Li^+ are not known, but determination of the neuronal effects of this ion may help to elucidate the regulation of neurotransmission and improve our understanding of the pathophysiological processes underlying affective disorders. Basic research on the action of Li^+ has focused on its effects on synaptic functions. Investigators have examined a variety of enzymes, ion channeles and pumps, receptors and second messenger systems for possible sites of action of lithium (17,18).

Apomorphine activates both D_1 and D_2 dopamine receptors (9-11). It has been reported that apomorphine induces stereotyped behaviour in mice, rats and dogs as well as in pigeons (12,13) apparantly pecking behaviour is centrally mediated and both D_1 and D_2 receptors are required to induce pecking (13).

According to these evidences and in order to study the interaction between serotonergic and dopaminergic transmission in the basal ganglia, we attempted to investigate the effect of acute administration of Li^+ and a new $5HT_{2A}$ antagonist AMI-193, 8-[3- (4-flourophenoxy) propyl]-1-phenyl-1,3,8- triazaspiro [4.5]decan-4-one (19) on pecking induced by apomorphine.

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MATERIALS AND METHODS

Pigeons of either sex weighing 200-400g were obtained from various local breeders. The animals were housed in groups of 3 or 4 in large cages, and maintained on a 12:12hr light : dark cycle. Standard food and water were freely available, except during the time of experiments.

All experiments performed between 9 am and 5 pm the birds were taken from their home cage and released in a testing cage (28×28×28) cm with three lateral walls covered with black paper sheets with dots (average of 5.5 dots/dmz), since dots elicit a strong pecking response to apomorphine injection (20). Animals were allowed to adapt for 30 min before the first injection. Observation started within one minute after the birds received apomorphine. The birds were all observed through a one way mirror by a single experimenter stationed in the room , thus minimizing disturbance of the pigeons , and apomorphine-induced pecking at the floor or side walls, pecking at their own plumage or toes , preening, drinking , wing- flapping , stretching one or both wings , body or head-shaking , yawning were observed. The number of pecking was counted by direct observation for 60 min after apomorphine injection . All drugs were injected into the leg muscle.

Apomorphine HCl ampules (TECHLOPHARM, Germany), (AMI-193) [8- [3-(4- Flourophenoxy) propyl]-1-phenyl]-1,3,8-triazaspiro [4.5] decan -4-one (Tocris Cookson, UK) $5HT_2$ antagonist ,Lithium chloride (Merck, Germany) was dissolved in distilled water and AMI-193 was dissolved in DMSO. The drugs were prepared immediately before use and were administered in a volume which contained Li^+ (240 mg/kg), AMI-193 (0.003 mg/pigeon). Apomor-phine was diluted in water to prepare suitable dose and metabisulfate was added as an antioxidant.

Statistical analysis: The data expressed as the mean ± SEM were analyzed by one way analysis of variance (ANOVA) and least significant difference (LSD) for multiple comparison. Differences with $p<0.05$ were considered statistically significant .

RESULTS

Injection of different doses of apomorphine (0.2-1.6 mg/kg) caused dose-dependent pecking behaviour in pigeons (Fig. 1). The maximum response was obtained with 1.6 mg/kg of the drug . Because of vomiting it was impossible to administer higher doses of apomorphine. Pretreatment of animals with $5HT_2$ antagonist (0.003 mg/pigeon) 15 minutes before apomorphine injection increased the pecking response induced by apomorphine (0.8 mg/kg intra-muscularly) (Fig. 2). Pretreatment of animals with single dose of lithium (240mg/kg) and single dose of $5HT_2$ antagonist (0.003 mg/pigeon) 15 minutes before apomorphine (0.8 mg/kg) decreased the pecking response induced by apomorphine (Fig. 3).

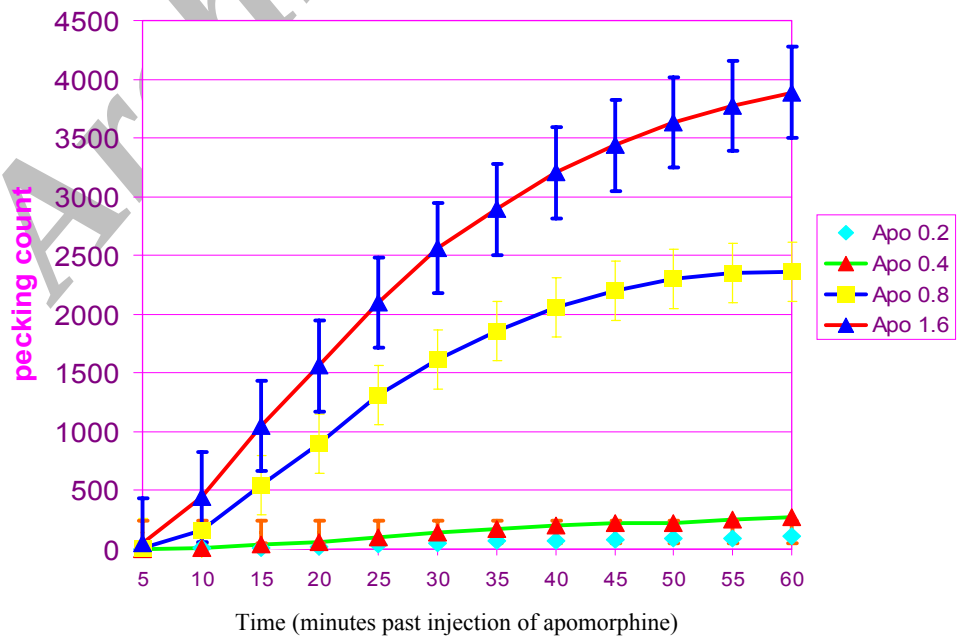

Fig. 1. Effects of Apomorphine (0.2 mg/kg) injection on pecking. Each line represents one case (n=5). The case marked with arrow was excluded from analyses

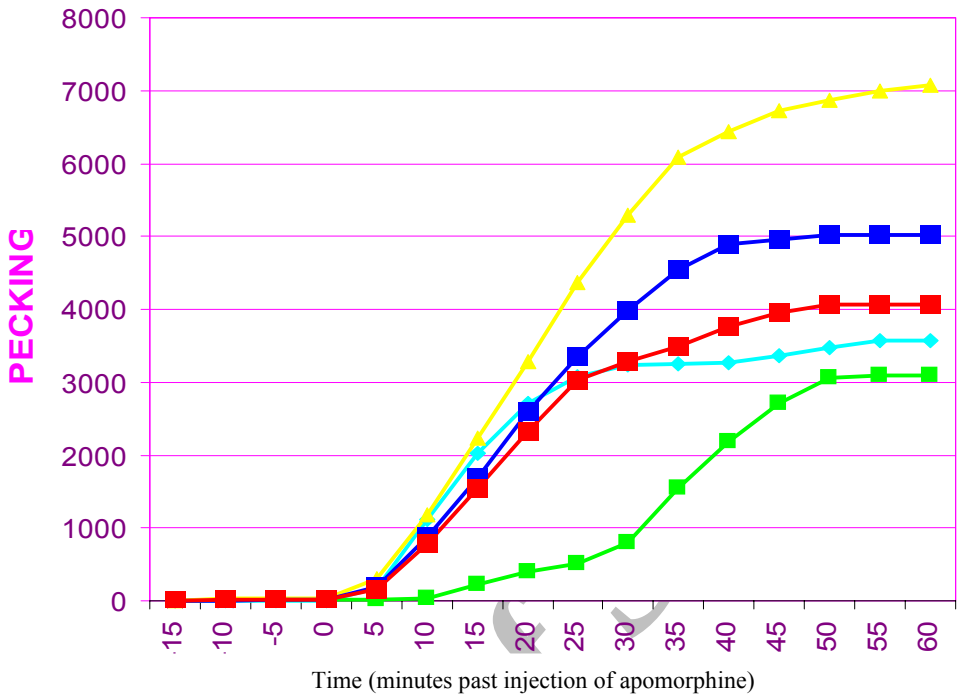

Fig. 2. Effects of 5HT2 plus Apomorphine (0.8 mg/kg) injection on pecking. Each line represents one case (n=5).

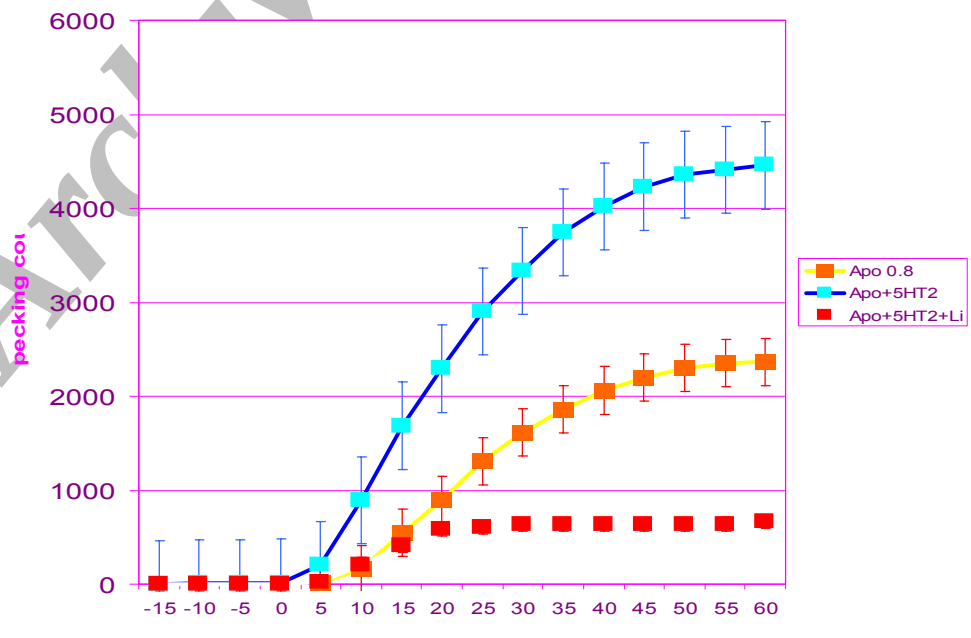

Fig. 3. Injection of Lithium (240 mg/kg) caused a significant ($p<0.05$) decrease in pecking caused by injection of apomorphine (0.8 mg/kg) alone and apomorphine + 5HT2 (0.003 mg/pigeon) after 60 minutes.

DISCUSION

We investigated the influence of acute Li^+ and a new $5HT_2$ antagonist on apomorphine induced pecking. Apomorphine induced pecking in pigeons dose dependently. Lithium produced no effect on pecking by itself. The $5HT_2$ receptor antagonist AMI-193 (19) increased the number of pecking response induced by apomorphine.

5-Hydroxytryptamine has been identified in nerve terminal varicosities within the striatum using high resolution autoradiographic techniques (21). 5-HT cotaining neurons originating from the midbrain raphe nuclei innervate both the SN and the VTA. Neuroanatomical studies have shown a high density of 5-HT-immunoreactive fibers in both the substantia nigra pars compacta and substantia nigra pars reticulata, in addition to the VTA (22). Serotonergic terminals make synaptic contacts with both dopamine –containing and non-dopamine-containing neurons in the substantia nigra pars compacta (SNc), substantia nigra pars reticulata (SNr) and VTA (22). Several 5-HT receptor subtypes have been shown to be present in the basal ganglia. High to moderate levels of 5-HT_{2A} and 5-HT_{2C} receptor binding sites are present in several forebrain areas including the basal ganglia and limbic system. Moderate levels of mRNA for both 5-HT_{2A} and 5-HT_{2C} receptors have been detected in the SN, whereas the VTA contains mRNA for 5-HT_{2C} but not 5-HT_{2A} receptors (22).

There has been some contraversy since past as to the function of 5-HT in the striatum since the neurotransmitter has been reported to have both excitatory and inhibitory effects (23). It has been shown that lesions of the medial raphe nucleus in rats produced an increase in striatal dopamine turnover, possibly mediated by an action through the SN (24-28). It means $5HT_2$ antagonist (AMI-193) inhibits the serotonin system and thus releases the dopamine from this inhibition in the Raphe-striatal pathway disinhibition of the dopamine system (29-34).

In support of an inhibitory role for 5-HT the antagonists cyproheptadine, methysergide and metergoline have been shown to potentiate the behavioural effects of apomorphine and amphetamine in rats (35). Therefore the present study was undertaken in an attempt to elucidate the effect of (AMI-193) on dopamine release and it seems it was acting on 5-HT receptors located on the nerve terminals of dopamine neurones in the striatum to modulate dopamine release.

Lithium and $5HT_2$ antagonist (AMI-193) decreased apomorphine induced pecking. It has been shown that Li inhibits the rise of cyclic AMP produced by the dopamine agonist in a synaptosomal prepration (36). On the other hand in certain cells, D_2 stimulation may activate phospholipase C which hydrolyses the membrane phospholipid,phosphatidyl inositol biphosphate (PIP_2) which again increases inositol triphosphate (IP_3) and intracellular calcium. Lithium treatment reduces the level of inositol in the brain through inhibition of inositol-1-phosphate (37) which could interfere with the resynthesis of PIP2 and thus influence the signalling mechanisms operating through the phsphoinositide system (38). Also Lithium has been reported to affect several serotonergic processes including synthesis, release and uptake of 5HT (38). As lithium increases serotonin release in the hippocampus (35) and short term administration of lithium increases the synthesis of 5-HT from tryptophan by the enzyme tryptophan hydroxylase, it also increases the transport of the 5-HT precurser, tryptophan in the hippocampus (38) and striatum (35). It has been shown that lithium administration increases tryptophan uptake into slices of rat striatum (35). As we know 5-Hydroxy-tryptamine (5-HT) has an inhibitory effect on dopamine release.As a result it is concluded that the inhibitory effect of Li and the new 5-HT_2 antagonist (AMI-193) on pecking induced by apomorphine is controlled by the mentioned mechanisms.

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