NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF SYNTHETIC ADRENERGIC AND RELATED COMPOUNDS ON RATS

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Abstract

The present study concerns neurochemical and behavioral effects of piperidine substituted 2 chloro 3, 4, dihydroxyacetophenone derivative compounds.Intraperitonial injection in rats significantly increases locomotors activity in open field experiment, anxiolytic effects of the newly synthesized compounds observed in light and dark apparatus were significantly increased, suggesting the anxiolytic effects of piperidine substituted 2 chloro 3, 4, dihydroxyacetophenone derivative. Moreover, stimulatory activity of compounds observed in Home cage apparatus which was significantly increased. Neurochemicals effects were observed by HPLC-EC, which showed that after administration of compound (I) (II) increased the level of dopamine in rats brain. Suggested that these piperidine substituted 2 chloro 3, 4, dihydroxyacetophenone derivative compounds may be effective as a drug for treatment of depression, enhancement of locomotion and stimulation. Further studies using animal's models of depression are required to establish these drugs.

Introduction

Adrenergic compounds are the chemical compounds which show their pharmacological and therapeutic effects by increasing or decreasing the activity of the various components of sympathetic division of the autonomic nervous system. (Blier P., 2001) when they produce effects similar to stimulation of sympathetic nervous activity are known as sympathomimetic or adrenergic stimulants. (Cryan 2000). Producing relaxation in shock, asthma, and normal functioning of heart, Adrenergic neurotransmitter's are biosynthesized in certain neurons of CNS known as Epinephrine (EP) & nor epinephrine (NE). Einat H, Clenet F, Shaldubina A, Belmaker R, Bourin M (2001). It was also known that if EP & NE like drugs was administered in the body, they have no effect on the CNS as it is unable to cross the blood brain barrier (BBB), Estrada-Camarena (2002), whereas only precursor can cross the BBB. Anxiety and depressive depend upon the secretion of these EP, NE and dopamine if the secretions are not normal which was observed are general in all regions of the world. Amashima T. (2003). They may be caused because of the financial problems in people suffering from infectious diseases, due to malnutrition, and where only a small percentage is allocated to health services (Howard, 2008). These disorders are very significant because of their financial cost. Previously some drugs were synthesized to remove these problems, and it was also observed that naturally occurring compounds are Catecholamines that have a catechol nucleus consisting β phenyl amine, on the Meta, and Para positions of the aromatic ring, and on the amino α, and β. Positions of the ethylamine side chain Sabyasachi Sircar (2007). Dopamine, adrenaline (epinephrine) and noradrenalin (nor epinephrine) released by the adrenal medulla of the adrenal glands in response to stress. Yamashima (2003). They were derived from the amino acid tyrosine. In the human body, the most abundant catecholamines,were epinephrine (adrenaline), nor epinephrine (noradrenalin) and dopamine, all of which are produced from phenylalanine and tyrosine. Various stimulant drugs are catecholamines. In comparison to dopamine, some drugs according to their structure activity relationship are known as dopamine agonists Shen, Howard (2008).These drugs bind to dopamine receptors in position of dopamine and directly stimulate those receptors. Sabyasachi Sircar (2007) some dopamine agonists are currently used to treat Parkinson's disease. These drugs can stimulate dopamine receptors even in someone without dopamine neurons. Malhotra A, Krilov LR (2001). In the present study the newly synthesized compounds (I) and (II) were very closely related to EP, NE and dopamine structurally.

Materials and Methods

Synthetic protocol of compounds

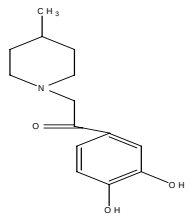

Fig.1. 1-(3,4-dihydroxyphenyl)-2-(4-methylpiperidine-1-yl)ethane-1-one.(I).

The two constituent reactants include: 1 methyl piperidine and 2 chloro 3, 4 dihydroxy acetophenone. These two reactants were dissolved separately in (15-20) ml of acetone, then mixed. Reaction mixture was stirred for 60 hours. at low temperature of 50-53 ^{o}C, process of reaction was monitored through thin layer chromatography, and the crude solid product was filtered and washed with acetone. The product thus obtained was purified through recrystallization by using methanol and ethyl acetate.

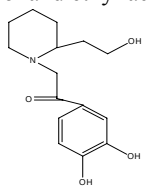

Fig.2. 1-(3,4-dihydroxyphenyl)-4-(piperidine-2-yl)butan-1-one.(II).

The two constituent reactants include: 2, Pyridyl ethanol and 2 chloro 3, 4 dihydroxy acetophenone. These two reactants were dissolved in acetone separately then mixed, reaction mixture was stirred for 73 hrs at low temperature of 50-52^{o}C. The process of reaction was monitored through thin layer chromatography, and the crude solid product thus obtained was purified through recrystallization by using methanol and ethyl acetate.

Animals: Locally bred male albino Wister rats weighing about 180 to 200gm on arrival purchased from animal house, research institute of Agha Khan University Karachi Pakistan were used throughout the experiments. The rates were housed individually in specially designed cages with saw dust cover floor in a quiet room, with free access to cubes of standard rats' food and water for at least, 3 to 4 days before starting the experiment, so that the rats could adapt themselves to the new environment.

Injection of compounds: Synthetic compounds injected to test group of rats, these compounds were dissolve in saline. Rats' were injected intraperitoneally (ip) with saline, compounds and parent compound.

Experimental protocol: Three groups of locally bred male albino Wister rats, weighing about 180 to 200g on arrival purchased from animal house, research institute of Agha Khan University Pakistan, each group contained seven rats, were used throughout the experiments. Rats were housed individually in specially designed cages with saw dust cover floor in a quiet room, with free access to cubes of standard rats' food and water for at least 3 to 4days before starting the experiment. After 4 days, synthetic compounds were injected to test group of rats, 30 mg/kg body weight, synthetic compound was dissolved in saline, control (vehicle) group (VG) was injected with saline and parent group (PG) was injected with 2chloro 3, 4 dihydroxy acetophenone. the test group (TG) was injected with (I) ,and (II) compounds. After ½ hour of injection activity was monitored for 5 minutes in, Home cage which was specially designed made up of Perspex (26×26×26cm) floor was soft due to saw dust, For the next 5 minutes the activity was monitored in the Open field, and the Open field apparatus consisted of box ,having square area (76×76cm) with walls of 42cm high, floor of the apparatus was divided by lines into 25 squares having equal size.

Last 5 minutes in light and dark environment. The light and dark apparatus was made up of two compartments and small passage was present between two compartment due to which rat could easily move to either compartment. After monitoring these activities, the animals returned to their cages. Rats were decapitated, after 7 hrs of injection, and then brains were collected very quickly from the cranial cavity within 30 seconds of decapitation. Whole brains and plasma, were collected and stored at low temperature (-70ºc) until analyzed by HPLC-EC detector. For HPLC-EC determination ,a 5µm shimpack ODS separation column of dimentations 4.5mm (internal diameter)×15 cm (length) was used, methanol (18%), octyl sodium sulphate (0.023%), and EDTA (0.05%) in 0.1M phosphate buffer were used as the solvent system. An operation potential of 0.8V (glassy carbon electrode vs./AgCL) was used for electrochemical detection.

Statistical analysis: Results were represented as mean, ±SD, Behavioral and neurochemicals data were analyzed by one- way ANOVA. Individual comparison was made by Newman-keuls test.

Results

Fig. 3a. shows effects of compound (I) on Home cage, of VG, TG, and PG, of rats. Statistical analysis by one- way ANOVA (df3,18) (f=11.214) (*p<0.01) shows that after administration of (I) in TG, the Home cage activity significantly increased as compared to VG and PG. male rats. Fig. 3b, shows effects of (II) compound on PG, TG. VG. treated rats, in Open field, Statistical analysis by one- way ANOVA (df3,18)(f=12.977) (**p<0.01).Individual difference by Newman–Keuls test shows that after administration (I) compound in TG the Novel environment behavior increases as compared to PG and VG and VG group of male rats**.** Fig 3c shows

effects of compound (I) on PG, TG. VG. Treated rats. Statistical analysis by one-way ANOVA (df2,18)(f=11.192)(*p<0.01).shows that after administration of compound (I) in TG, the entries in light portion increased as compared to VG and PG, group of male rat. Fig 3d shows effects of compound (I) on PG, TG. VG.

Treated rats. Statistical analysis by one-way ANOVA (df2,18)(f=14.213)(**p<0.01).shows that after administration of compound (I) in TG, the rats spend their more time in light portion as compared to VG and PG, group of male rats.

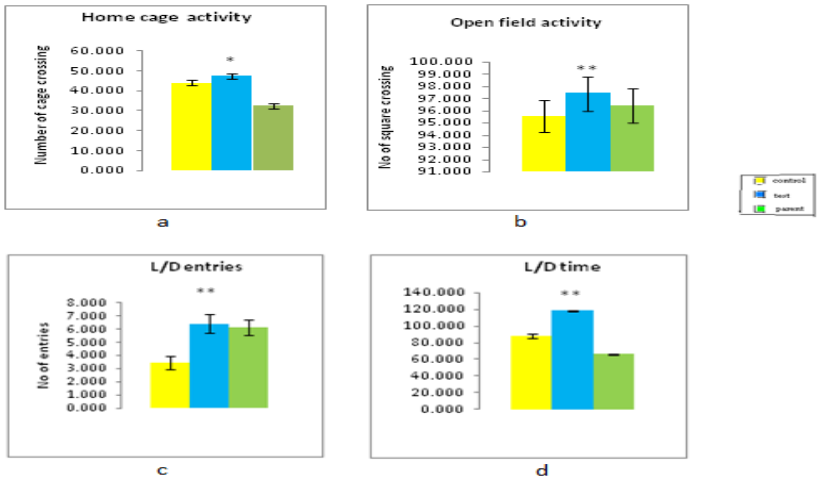

Fig.3. Effect of compound (I) on (a) Home cage activity, (b) Open field activity,(c) light and dark activity(entries) (d) light and dark time respectively. values are mean ±SD,(n=7)significant difference by Newman Keuls test;*p<0.01 from Home cage ,p<0.01from Open field,**p<0.01from light and dark entries, **p<0.01from light and dark time following one-way ANOVA.**

Fig 4e. shows effects of compound (II) on Home cage, of VG, TG, and PG, of rats. Statistical analysis by one-way ANOVA (df3,18) (f=15.317) (*p<0.01) shows that after administration of compound (II) in TG, the Home cage activity significantly increased as compared to VG and PG. male rats. Fig 4f, show effect of compound (II) on PG, TG. VG, treated rats, in Open field. Statistical analysis by one- way ANOVA (df3,18)(f=13.309)(*p<0.01).Individual difference by Newman –Keuls test shows that after administration of (II) in TG the Novel environment behavior increased as compared to PG and VG and VG group of male rats Fig 4g, show effect of (II) compound on PG, TG. VG. Treated rats. Statistical analysis by one-way ANOVA (df2,18)(f=12.204)(*p<0.01).shows after administration of compound (II) in TG, the entries in light portion increased as compared to VG and PG, group of male rat. Fig 4h, shows effect of compound (II) on PG, TG. VG. Treated rats. Statistical analysis by one-way ANOVA (df2,18)(f=15.213)(**p<0.01).shows that after administration of (II) in TG, the rats spend their more time in light portion as compared to VG and PG, group of male rats.

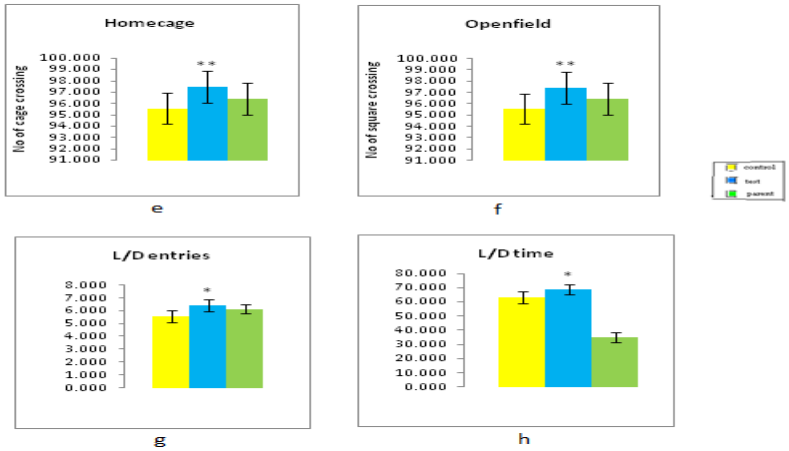

Fig. 4. Effect of synthetic compound (II) on (a) Home cage activity, (b) Open field activity,(c) light and dark activity(entries) (d) light and dark time respectively. values are mean ±SD,(n=7) significant difference by Newman Keuls test;p<0.01 from Home cage ,**p<0.01from Open field,*p<0.01from light and dark entries, *p<0.01from light and dark time following one-way ANOVA.**

Fig. 4. shows the effect of compound (I) on concentrations of dopamine,5HIAA, HVA, and 5HT, in the whole brains of PG, TG, and VG., Statistical analysis by one-way ANOVA(df2,18) (f=29.403)(p<0.01)Dopamine.(df2,18)(f=0)(p>0.05)5HIAA.(df2,18)(f=12.32)(p<0.01)HVA.(df2,18)(f=0)(p>0.05) 5HT.Individual difference made by Newman Keuls test. Fig 5, I, shows that after administration of compound (I) the concentration of dopamine was increased in TG, as compared to PG, and VG, in whole brains of rats. Fig 5,k,. and fig 5,l, show that the effect of compound (I) on concentration of 5HIAA,and 5HT in TG, PG, VG was same and no change was found in 5HIAA and 5HTin the whole brains of rats. But Fig 5,j,.shows that after administration of compound (I), the concentration of HVA in the whole brain of TG was increased as compared to, PG, and VG, groups of rats.

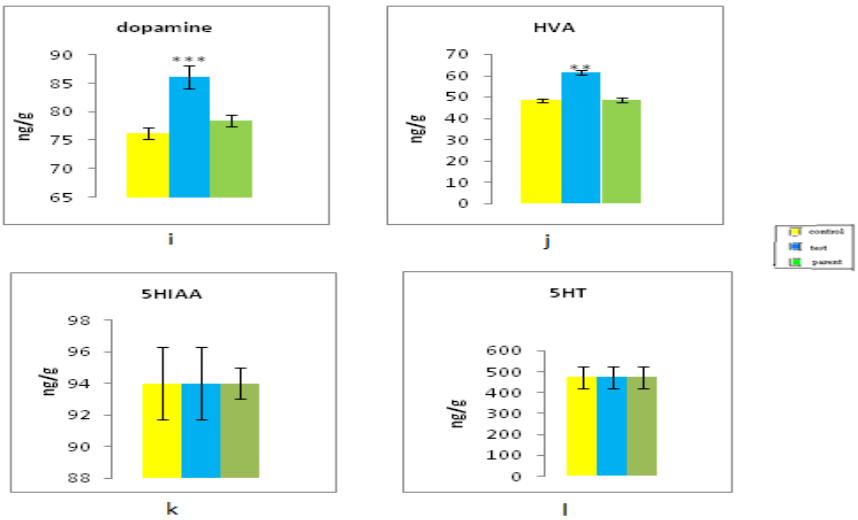

Fig.5. Effect of 30mg/kg body weight of synthetic compound (I) on (i) Dopamine, (j) HVA,(k) 5HIAA,(l) 5HT,respectively. values are mean ±SD,(n=7)significant difference by Newman Keuls test;*p<0.01 from Dopamine,**p<0.01fromHVA,p>0.05from5HIAA, p>0.05from 5HT, following one-way ANOVA.**

Fig 6. shows the effect of compound (II) on concentrations of dopamine,5HIAA, HVA,and 5HT, in the whole brains of PG, TG, and VG.,group of rats, Statistical analysis by one-way ANOVA(df2,18)(f=21.23)(p<0.01) Dopamine.(df2,18)(f=0) (p>0.05)5HIAA.(df2,18) (f=20.13) (p<0.01)HVA.(df2,18) (f=0) (p>0.05) 5HT.Individual difference made by Newman Keuls test.fig 6m, shows that after administration of (II) concentration of dopamine was increased in TG, as compared to PG, and VG, in whole brains, in fig 6o,.and In case of fig 6 p show that the effect of (II) on concentration of 5HIAA, and 5HT in TG, PG, VG was same and no change was found in 5HIAA and 5HT in the whole brains. But fig 6n,.shows that after administration of (II), the concentration of HVA in the whole brains of TG was increased as compared to, PG, and VG.group of rats.

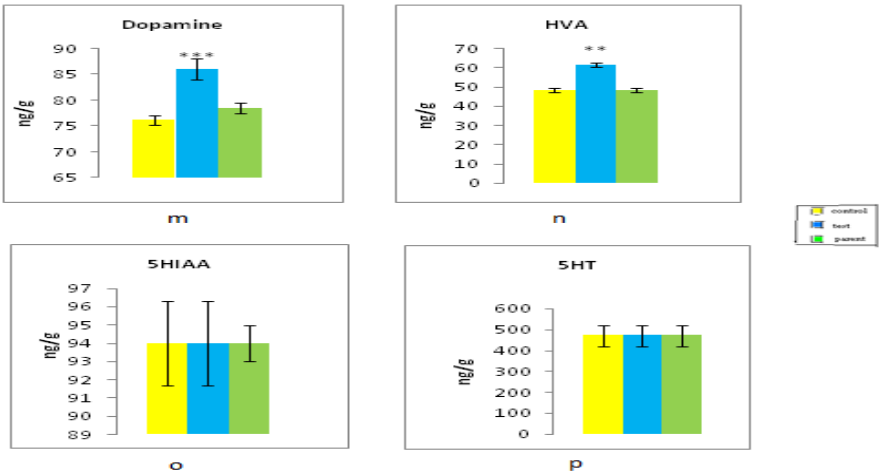

Fig.6. Effect of 30mg/kg body weight of synthetic compound (II) on (m) Dopamine, (n) HVA,(o) 5HIAA,(p) 5HT,respectively. Values are mean ±SD,(n=7)significant difference by Newman Keuls test;*p<0.01 from Dopamine,**p<0.01fromHVA,p>0.05from5HIAA, p>0.05from 5HT, following one-way ANOVA.**

Discussion

Previous studies show that if parent structure for several of the sympathomemitic drugs is substituted by β phenylamine, on the meta, and para positions of the aeromatic ring, and on the amino α, and β. positions of the ethylamine side chain,it not only authorizes the mechanism of sympathomemitic events but also the receptor selectivity of the drug Elizabeth Scott, M.S. 2007. For the direct acting sympathomemitic amines. Ideal activity was seen in β phenyl ethylamine derivatives ,containing OH group in the meta and para positions of the aromatic ring ,and on the amino, α, and β positions of the ethylamine side chain, Allard *et al* 2004 which influenced not only the mechanism of sympathomimetic actions but also the receptor selectivity of the drug (Baldo *et al.,* 2004). For the direct acting sympathomimetic amines ,maximum activity was seen in β phenylethylamine derivatives containing OH group in the meta and para positions of the aromatic ring ,and a β hydroxyl group of the correct stereochemical configuration on the ethylamine portion of the molecule Barrot *et al* 2002. Such structural features were seen in the prototypical direct acting compounds, NE, EP, dopamine and proterenol .A critical factor in the interaction of adrenergic agonists with their receptors i (Berton *et al.,* 2004). Direct acting sympathomimetics, that show chirality by high caliber of the presence of a β hydroxyl group (phenyl ethyl amines)invariability display high stereo selectivity in producing their agonist effects, that was one enantiomeric form of the drug ,has greater attraction for the receptor than the other selection has. Sabyasachi Sircar (2007). This structural activity relationship which was proved by the previous experiment. In comparison to dopamine, some drugs according to this structure activity relationship are known as dopamine agonists. These drugs bind to dopamine receptors in position of dopamine and directly stimulate those receptors. Some dopamine agonists are currently used to treat Parkinson's disease. These drugs can stimulate dopamine receptors even in someone without dopamine neurons. People with Parkinson's disease drop neurons that contain dopamine. Bjornson CL, Johnson DW (2008). To fight this disease, the body produces additional dopamine receptors on other neurons. Indirect agonists are not very effective in treating the disease since they depend on the presence of dopamine neurons. In comparison, direct agonists are more effective because they stimulate dopamine receptors even when the dopamine neurons are lost (Sun, *et al,* 2002). *(*http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T4S-4JKHPBT-1&_user=3415223&_coverDate=06%2F15%2F2006&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1710440039&_rerunOrigin=scholar.google&_acct=C000060484&_version=1&_urlVersion=0&_userid=3415223&md5=1c5690167dac17e74bdf065aa29e6f4b&searchtype=a - bbib100). In the present study, considering the fact on the basis of literature that substituted phenyl ethyl amines derivatives of piperidine induce brain catecholamines. it was of profound importance to investigate. (I) and (II) ,newly synthesized derivatives of piperidine on brain dopamine in rats assuming that these (I),(II),results shown in Fig 6m, and Fig 5i increased concentrations in dopamine.. fig 6n, and Fig 5j shows an increased concentration in HVA, except 5HTand 5HIAA.The enhancement of both dopamine and its metabolite HVA following the administration of this derivative suggests an increased turnover of dopamine due to an increase in the activity of particularly rate –limiting catecholamine synthesizing enzyme tyrosine hydroxylase. Berton *et al.,* 2004).

Conclusion

In the present neurochemical studies showed significant increase in the concentration of dopamine in test group of rats and also increase was monitored in HVA this was due to the degradation of dopamine in the brain. But in case of 5HT, and 5HIAA it did not change. It was conclude that these two (I), and (II) drugs may be effective for the treatment of depressions and anxiety.

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