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Original Article

Assessment of Role of Dopamine in Medial Amygdala on Body Weight, Metabolic Profile and Lipid Risk Factors in Albino Wistar Rats

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ABSTRACT

Background and Aim: The present study was conducted to assess the role of dopamine in amygdala in the body weight, blood glucose, lipid profile and lipid risk factors in albino Wistar rats. **Methods:** A total of 16 albino Wistar rats were taken for the study and were divided into medial amygdalar group and control group with 8 rats in each group. Stereotaxic cannulation was performed and dopamine was injected into the medial amygdalar nuclei. Blood sample was obtained for estimation of metabolic parameters (blood glucose and lipid parameters) before and after dopamine injection, by computerized metabometer and autoanalyzer. **Results:** Following dopamine injection, there was decrease in body weight, blood glucose, lipid profile and lipid risk factors in medial amygdala group compared to control group. There was positive correlation of body weight with blood glucose, triglyceride, very low-density lipoprotein and atherogenic index. **Conclusion:** Decrease in body weight, blood glucose, lipid profile and lipid risk factors induced by dopamine in medial amygdalar group could be linked to reduction in cardiometabolic risks in these rats. Reduction in body weight could be the key element in inducing reduction of cardiometabolic risks.

Key words: Dopamine, Cardiometabolic risk, Medial amygdalar nucleus, Stereotaxy, Wistar rats, Lipid risk factors.

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INTRODUCTION

Obesity is a major health problem of the society and current WHO estimate suggests that over 1 billion people are overweight and over 300 million people are obese.[1] It is associated with various disorders like hypertension, diabetes, hyperlipidemia and hypothyroidism. However, the exact mechanisms that cause obesity are not known and therefore the anti-obesity neural mechanisms have not been fully elucidated. Whether the brain areas like lateral hypothalamus (LH), ventromedial hypothalamus (VMH), nucleus septal medial is (NSM), arcuate nucleus (AR) and amygdala directly influence the feeding behavior and body weight control or they alter the sympathetic activity and thereby alter energy intake and expenditure for control of obesity is not clear.

Food intake is controlled by a complex system of both central and peripheral signals that interact to alter the individual response to feeding. The peripheral regulation includes the satiety signals and the central control is by various neurotransmitters acting on the different brain areas. Para-hypothalamic areas such as nucleus accumbens, nucleus caudatus, nucleus septal lateralis and amygdala have been suggested to play a significant role in the regulation of ingestive behaviors. In addition, there are many projections to and from the brainstem, cortical areas and reward pathways, which modulate food intake.

Amygdala, the major part of the older brain, the limbic system is the seat of basic instincts, memory and emotions.[2] Earlier we have reported dopamine as an anorexigenic and anti-dipsogenic neurotransmitter in hypothalamic and parahypothalamic nuclei in experimental models.[3-6] Dopamine has also been reported to be neurotransmitter in amygdala involved in control of neuro-visceral functions including ingestive behaviours. Emotion has been reported to be linked to cardiometabolic risks.[7] However, role of amygdala on cardiometabolic profile, especially on the lipid risk factors has not been studied yet. Therefore, in the present study we assessed the role of dopamine injected into amygdala on cardiometabolic functions including lipid risk factors in experimental animal models.

MATERIALS AND METHODS

Animals

This was an interventional experimental study conducted in the Animal Research Laboratory of Physiology department of Jawaharlal Institute of

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Postgraduate Medical Education and Research (JIPMER), Puducherry, India. The experimental protocol was approved by Postgraduate Research monitoring Committee (PGRMC) JIPMER, Puducherry and the Animal Ethics Committee of Pondicherry University. After obtaining Animal Ethics Committee approval, 16 albino rats of Wistar strain of 3 to 6 months old weighing 190-250 gm was obtained. The rats were housed in individual cages in a paddy husk floor with standard rat chow diet in the Animal Research Laboratory of Physiology department for 10 days for habituation. Food and water were available *ad libitum* in a temperature-controlled environment.

Groups

They were divided into 2 groups as follows.

Medial amygdala group (n=8): Eight male rats with injection of dopamine into medial amygdala.

Control group (n=8): Eight male rats without injection of dopamine into medial amygdala group.

Procedures

Stereotaxy and Assessment of Ingestive Behaviours

Stereotaxic cannulations were performed individually in each rat for intranuclear injections in the JIPMER Animal Research Laboratory, Department of Physiology, following the standard procedures and using the co-ordinates of brain atlas (Konig and Klippel).[8] It was done under complete anesthesia following CPCSEA guidelines. Ketamine was used as anesthesia during the procedure. Following recovery from stereotaxy, the food intake (FI), the water intake (WI) and activity of the animals were estimated by using computerized metabometer (Physiocage, USA).

Injection of Dopamine

After complete recovery from cannulation, 2 µg of dopamine was injected and the effects of dopamine on cardiometabolic profile (heart rate, food intake, water intake, body weight, blood glucose and motor activities) utilizing computerized metabometer (as we have seen 2 µg of dopamine as the maximum stimulating dose in our previous studies).[4-6]

Blood Collection

Two ml of blood was collected from retro-orbital plexus (wherever feasible) before and after injection of dopamine into medial amygdalar nucleus, for biochemical estimations. It was done under complete anesthesia following CPCSEA guidelines. Rats were anesthetized by inhalational Isoflurane anesthesia. Blood glucose estimation was done using strip (dip-stick) method, using (Accu Chek Glucometer, Aviva, India). Lipid profile estimation was done in JIPMER Biochemistry department Laboratory, by Auto-analyzer.

Statistical Analysis of Data

All data were recorded as mean±SD. Data across the group (comparison of medial amygdalar group with control group) were compared by unpaired t test. Correlation of body weight with food and water intake, blood glucose and lipid parameters of control and medial amygdalar group was performed by Pearson correlation analysis. P value <0.05 was considered as statistically significant.

RESULTS

Comparison of body weight, food intake, water intake, activity and blood glucose in rats prior to dopamine injection

The mean body weight of the rats in control group and medial amygdalar group did not differ significantly. In the baseline, there was no significant difference in food and water intake, blood glucose and activity between the groups (Table 1).

Comparison of lipid profile and lipid risk factors in rats prior to dopamine injection

There was no significant difference observed in total cholesterol (TC), Triglyceride (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), high density lipoprotein cholesterol (HDL-C), TC/HDL, TG/HDL, LDL/HDL and atherogenic index (AI) in rats between control group and medial amygdalar group (Table 2).

Effect of injection of 2µg of dopamine on body weight, food intake, water intake, activity and blood glucose

Body weight was significantly decreased (P<0.05) in medial amygdalar group compared to control group after dopamine injection. Food intake and water intake was significantly decreased (P<0.001) in medial amygdala group compared to control group following injection of dopamine.

There was significant difference observed in activity in medial amygdalar group (P<0.05) compared to control group. Following dopamine

Table 1: Comparison of body weight, food intake, water intake, activity and blood glucose in rats of control group and medial amygdala group prior to dopamine injection.

Parameters	Control Group (n=8)	Medial Amygdala Group (n=8)
Body weight (g)	225.92±9.04	223.77±10.50
Food intake (g)	14.88±3.18	14.68±3.21
Water intake (ml)	23.15±2.88	22.84±3.08
Activity (No. of movement/24h)	46133.56±5861	46112±5697.16
Blood Glucose (mg%)	125.23±12.62	123.±14.18

The values are expressed as mean±SD. Data was analyzed by unpaired t test.

Table 2: Comparison of lipid profile and lipid risk factors in rats of control group and medial amygdala group prior to dopamine injection.

Parameters	Control Group (n=8)	Medial Amygdala Group (n=8)
TC (mg/dL)	58.42±5.62	61.42±9.62
TG (mg/dL)	93.14±9.63	87.14±15.91
LDL-C (mg/dL)	14.14±2.85	15.85±6.26
VLDL-C (mg/dL)	18.71±2.07	16.42±3.10
HDL-C (mg/dL)	35.71±4.68	32.94±4.56
TC/HDL	1.64±0.38	1.85±0.55
TG/HDL	2.60±0.58	2.68±0.50
LDL/HDL	0.40±0.15	0.48±0.19
Atherogenic index	0.41±0.12	0.42±0.11

The values are expressed as mean±SD. Data was analyzed by unpaired t test.

TC: Total cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; VLDL-C: VLDL-cholesterol; AI: Atherogenic index: log10 (TG/HDL-C).

injection, blood glucose was significantly decreased in medial amygdalar group (P<0.01) compared to control group (Table 3).

Effect of injection of 2µg of dopamine on lipid profile and lipid risk factors

The lipid parameters such as TC (P<0.05), TG (P<0.01), LDL-C (P<0.05), VLDL-C (*p*<0.01) and HDL-C (P<0.05) were significantly reduced in medial amygdala group compared to control group after injection of dopamine. Atherogenic index (AI) was significantly increased in medial amygdala group compared to control group (Table 4).

Correlation of body weight with food and water intake, blood glucose and lipid parameters of control and medial amygdala group

There is a positive correlation of body weight with food intake (r =0.452, P=0.009), water intake (r =0.370, P=0.022), blood glucose (r =0.352, P=0.025), TG (r =0.373, P=0.021), VLDL-C (r =0.473, P=0.008) and AI (r =0.365, P=0.043) in medial amygdalar group. There was no significant correlation of body weight with any of the parameters in the control group (Table 5).

Table 3: Effect of injection of 2µg (in 1 µl of normal saline) of dopamine on body weight, food intake, water intake, activity and blood glucose in rats of medial amygdala group. Control rats received equal volume of normal saline, without dopamine.

Parameters	Control Group (*n*=8)	Medial Amygdala Group (*n*=8)
Body weight (g)	225.45±10.26	216.86±9.70*
Food intake (g)	15.291±2.98	9.67±2.51***
Water intake (ml)	23.00±3.11	10.67±2.08***
Activity (No. of movement/24h)	46107.86±5690.11	40312±8697.16*
Blood Glucose (mg%)	126.83±10.57	114.67±4.16**

The values are mean±SD. Data was analyzed by unpaired *t* test.
P values less than 0.05 was considered significant.
* P < 0.05; ** P < 0.01; ***P<0.001

Table 4: Effect of injection of 2 µg (in 1 µl of normal saline) of dopamine on lipid profile and lipid risk factors in rats of medial amygdalar group. Control rats received equal volume of normal saline, without dopamine.

Parameters	Control Group (*n*=8)	Medial Amygdala Group (*n*=8)
TC (mg/dL)	67.57±9.30	59.33±9.55*
TG (mg/dL)	64.14±10.55	54.33±4.93**
LDL-C (mg/dL)	11.87±3.20	9.16±2.21*
VLDL-C (mg/dL)	27.14±9.20	19.56±8.10**
HDL-C (mg/dL)	33.57±6.50	28.66±5.23*
TC/HDL	2.01±0.24	2.07±0.25
TG/HDL	1.91±0.32	1.89±0.34
LDL/HDL	0.32±0.13	0.33±0.11
Atherogenic index	0.27±0.09	0.31±0.0.08*

The values are mean±SD. Data was analyzed by unpaired t test.

P values less than 0.05 was considered significant.

* P < 0.05; ** P < 0.01;

TC: Total cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; VLDL-C: VLDL-cholesterol; AI: Atherogenic index: log10 (TG/HDL-C).

Table 5: Pearson correlation analysis of body weight with food and water intake, blood glucose and lipid parameters of control and medial amygdala group.

Parameters	Control Group		Medial Amygdala Group	
	r	P	*r*	P
Food intake	0.031	0.552	0.452	0.009
Water intake	0.103	0.704	0.370	0.022
Blood glucose	0.031	0.552	0.352	0.025
TC	0.103	0.704	0.310	0.056
TG	0.026	0.922	0.373	0.021
LDL-C	0.006	0.983	0.270	0.083
VLDL-C	0.035	0.487	0.473	0.008
AI	0.016	0.598	0.365	0.043

P values less than 0.05 was considered significant.

TC: Total cholesterol; HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol; TG: Triglyceride; VLDL-C: VLDL-cholesterol; AI: Atherogenic index

DISCUSSION

In the present study, we found significant decrease in body weight, food and water intake following administration of 2µg of dopamine into medial amygdala compared to control rats. Thus, these findings indicate that dopamine could be a neurotransmitter for reduction in body weight in medial amygdala. Though hypothalamic areas (LH, VMH, AR) and extrahypothalamic areas (NSM, VM) are known to influence food intake and body weight,[9,10] recently para-hypothalamic areas such as nucleus accumbens, nucleus caudatus, nucleus septal lateralis and amygdala have been suggested to play a significant role in the regulation of ingestive behaviors.

Neuronal pathways between these nuclei are organized into a complex network in which orexigenic and anorexigenic circuits influence food intake and energy expenditure.[2] Firmly established pathways involving orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) (NPY/AgRP); and the anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART), (POMC/CART) neurons project from hypothalamic nuclei to different nuclei of amygdala through limbic-hypothalamic connections.[11] From the findings of the present study, we propose that dopamine could be a component of these anorexigenic neurotransmitters in meso-limbic system.

Though we have reported catecholamines including dopamine injected into mesolimbic structures to inhibit food and water intakes,[12-14] till date it is not clearly known if reduction in body weight is secondary to reduction in food intake or is a primary phenomenon of anti-ponderostatic role of dopamine in these nuclei. In the present study, the decrease in body weight was quite significant, which usually should not be secondary to reduction in food intake in such a short span of 72 hrs of injection of dopamine. Therefore, it is likely that body weight reduction could be due to direct effect of dopamine on body-weight control mechanism.

In the present study, there was profound decrease in blood glucose, lipid levels and lipid risk factors following central administration of 2µg of dopamine in medial amygdalar group compared with that of control

group. Thus, it appears that dopamine in medial amygdalar group has significant influence on control of fat and carbohydrate metabolisms. Interestingly, there was a significant correlation of blood glucose, TG, VLDL and AI with body weight as demonstrated by Pearson's correlation analysis, which indicates that the decrement in body weight, blood glucose and lipid profile and lipid risk factors induced by dopamine in medial amygdalar group could be linked to reduction in cardiometabolic risks in these rats. Also, it appears that reduction in body weight gain could be the primary factor that eventually decreases the cardiometabolic risks, following injection of dopamine into medial amygdala.

The findings of the present study indicate the role of amygdala on cardiometabolic functions in animal models. This is the study of its first kind to report the role of medial amygdala on metabolism, especially the lipid metabolism and the lipid risk factors. Future studies may further explore the role of amygdalar nuclei in the regulation of cardiometabolic profile in human beings, which may help in designing the strategies in elucidating impact of medial amygdala in reducing CV risks.

CONCLUSION

It was concluded that injection of dopamine into medial amygdala leads to reduction in food intake and body weight, animal activity, decrease in blood glucose and lipid risk factors. Thus, it appears that medial amygdala plays an important role in regulation of body weight and cardiometabolic functions and dopamine could be a crucial neurotransmitter in mediation of this cardiometabolic functions.

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ABBREVIATIONS

LH: Hypothalamus; **VMH:** Ventromedial Hypothalamus; **NSM:** Nucleus Septal Medialis; **AR:** Arcuate Nucleus; **TC:** Total Cholesterol; **TG:** Triglyceride; **LDL-C:** Low Density Lipoprotein Cholesterol; **VLDL-C:** Very Low Density Lipoprotein Cholesterol; **HDL-C:** High Density Lipoprotein Cholesterol; **NPY:** Neuropeptide Y; **AgRP:** Agouti-Related Protein; **POMC:** Pro-Opiomelanocortin; **CART:** Cocaine and Amphetamine-Related Transcript.

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