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Volumetry of Brain of Rat Following Methadone and Buprenorphine Administration

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Abstract: This study was performed to determine the probable effects of Buprenorphine and Methadone on brain volume of male rats. In this study 15 Wistar male rats were selected and divided into three groups randomly (n = 5).The first group was administrated 0.5 mg kg^{-1} methadone intraperitonealy for 15 days. Second group was administrated 30 ng buprenorphine intraperitonealy for 15 days. No injection was done on controls. Soon after the last injection, the animals were anaesthetized deeply, and then the brain dissected carefully and put in 7% buffered formaline solution. Following fixation brains were embedded in 3.5% agar and 1 mm coronal slices were prepared by means of a tissue slicer . Afterward using Cavalieri's principles, the volumes of the brains were calculated by point counting. Results were not showed any significant changes in volume of rat's brain following methadone and buprenarphine administration on the brain volume in rats.

Key words: Buprenorphine, methadone, brain, volume, Cavalieri's principles

INTRODUCTION

Nowadays in treatment of addiction agonist and partial agonist of opioid receptors are used (Katzong, 2001; Barchfeld and Medzinradsky, 2002; Blasing and Hers, 2000) and methadone and buprenorphine has got wide application (Katzong, 2001). Methadone is a full agonist of opioid receptor with half-life of about 18 h (Katzong, 2001; Barchfeld and Medzinradsky, 2002; Blasing and Hers, 2000; Johnson and Chutuape, 2000). Buprenorphine is a partial agonist of opioid receptors with half-life of about 1.2-7.2 h. Both of them metabolized in liver and excreted through kidneys (Katzong, 2001; Barchfeld and Medzinradsky, 2002; Blasing and Hers, 2000; Johnson and Chutuape, 2000). These drugs have a high absorbability through intra venous and oral route and they reaches high blood concentration very quickly. They get attached with plasma proteins and spread in whole body and reach specific targets in Central Nervous System (CNS) (Johnson and Chutuape, 2000).

Because high numbers of receptors are in brain, it is the main target for drug effect and adverse effect. The effect of drugs on brain have been studied in different aspects. One of these aspects is the effect of methadone and buprenorphine on brain volume.

In the study conducted by Donass and Anroos (1998) on brain mass of adult male addicted to heroine showed increment in CSF volume. Teo *et al.* (2000) showed increase in hypophysis gland mass due to opioid addiction. Prazos and Fischer (2002) demonstrated decrease in brain mass and its ventricles in heroine addicted individuals.

Shohani (2000) showed no changes in brain volume following morphine injection in rats. In all above studies stereological methods were used. With tending the above results into account and the fact that methadone and buprenorphine are opioid depravities and are used in a wide range as an analgesic, this study was performed to asses changes in brain mass of adult male rats following methadone and buprenorphine administration.

MATERIALS AND METHODS

Fifteen male adult Wistar rats were randomized into three groups (each including 5 rats) weighing 200±50 g. They were prepared from Razmjoo Moghaddam Institute of Zahedan Medical Sciences University, Iran. All animals lived 12 h in dark and 12 h in light under 22±2°C in separated cages. Food and water were made easily available for them. First and second groups were administrated 0.5% mg kg^{-1} methadone and 30 ng buprenorphine intraperitonealy for 15 days. There was not any administration on the controls. Afterward all animals were anesthetized by ether and their brains were fixed in modified Lillie's solution for 72 h.

Morphometry: Brains washed by distilled water and embedded in 7% agar. Agar blocks contain brains sliced by means of a tissue slicer and a histopathological knife into 1 mm slices, slices put on the table and a gird

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contains crosses threw randomly on the slices and hitted points with gray matter and white matter counted and recorded. The area around a point was measured and put in below equation and estimate volume of the brain (Mayhew, 1988; Michel and Cruz-orive, 1988):

$$V = \Sigma P. A (p). t$$

RESULTS AND DISCUSSION

Results showed that there is not significant difference in gray matter, white matter and total brain volume due to administration of methadone and buprenorphine (Table 1). The mean total brain volume, white matter and gray matter for the methadone group were estimated 14.3±1536.4 , 15.83±957.2 and 602.8±15.83 mm^3 (Table 1). The mean total brain volume, white matter and gray matter of buprenorphine group were estimated 15.83±1560, 14.3±934 and 14.3±602 mm^3 (Table 1) . The mean total brain volume, white matter and gray matter for the control group were 12.63±1557, 12.63±945 and12.63±619.2 mm^3 (Table 1). Opioids are the most powerful known pain relievers. Their use and abuse date back to antiquity. The pain reliving and exphoric effects of opiods were known to Soumerians [4000] and Egyptians [2000] BC (Rehman and Khoromi, 2003).

Various opioid receptors exist in the mammalian, CNS, namely mu, kappa, sigma, delta and epsilon.

These receptors are located in the brain mostely in the pereaqueductal gray, spinal cord, peripheral nerves system,adrenal medulla ganglia and gut (Johnson and Chutuape, 2000).

Highest number of receptors is in brain so it's the main target for drug effects and advers effects (Herz, 1998).

In utero opioid exposure consistently has shown a decrease in nucleic acid synthesis and protein production in the brain suggesting that overall brain growth is compromised (Rehman and Khoromi, 2003). Effects on neurotransmitter concentrations and production have not been confirmed (Rehman and Khoromi, 2003). Cerebrospinal fluid (csf) space enlargement has been demonstrated in substance-related disorders like alcohol and cocaine and opioid dependence (Donas and Anroos, 1998). Experimental animal studies showed a reduction in shape and size of mesolimbic dopaminergic neurons after chronic morphine administration other studies indicated a change of neurofilament and glial fibrillary acid proteins after chronic opiate administration (Kosten and Kleber, 2000). Further more, frequent over dosing and toxicological effects of street heroin May lead to csf space enlargement in opioid dependence (Donas and Anroos, 1998).

Table 1: Mean values of total brain, white matter and gray matter in three groups

Case NO.	∑P	CE	Total brain	White matter	Gray matter
Methadone group					
M_1	129	0.04	1548	948	600
M_2	131	0.04	1572	840	732
M_3	135	0.04	1620	982	638
M_4	129	0.03	1548	1020	528
M_5	126	0.04	1512	996	516
Buprenorphine group					
B_1	132	0.06	1584	960	624
B_2	135	0.05	1620	972	648
B_3	125	0.03	1500	984	516
B_4	120	0.03	1440	816	624
B_5	128	0.04	1538	938	600
Control group					
C_1	130	0.03	1560	984	576
C_2	134	0.03	1608	1008	600
C_3	128	0.04	1536	888	648
C_4	132	0.04	1584	936	684
C_5	125	0.03	1500	912	588

Methadone is a full agonist of opioid receptors and buprenorphinis a partial agonist of opioid receptors both drugs are opioid and have the effect of these categories (Katzong, 2001; Barchfeld and Medzinradsky, 2002; Blasing and Hers, 2000; Johnson and Chutuape, 2000).

Although the full spectrum of physical damage that drug of abuse can cause is not ducumentable, one thing is certain: The effect of brain development is the most critical and most studied effect.

The two braud classes of brain insult are as follows:

- Damage can occur during cytogenesis and cell migration
- Damage can occur during brain growth and differentiation (Rehman and Khoromi, 2003).

This fact that methadone and buprenorphine are opioids and have effect on brain it is probable that these drugs being effective on brain volume this study performed and its results showed no significant effect on total brain volume, gray matter and white matter with this period and dosage. Shohami (2000) reported that was established for evaluation the effect of morphine on cerebellar cortex volume of rat with cavalieri method, found that there was no significant change in cerebellar volume , probably due to restricted duretion of the study. Using CT scan, Baker and Harding (1999) showed that chronic addictive use of alcohol and opium simultaneously, had no effect on brain volume. May be this finding can be attributed to inhibitory effect of alcohol on some of opioid effects .

Whit the aid of computerized tomography, Cascell (1991) found that opioids had no effect on brain cortex volume or cerebral ventricles size.

This finding can also be due to this fact that the sample was gathered from the population that had no nutritional problem. The results of these studies are compatible to the present results. May be due to kind, dosage and duration of administration of the used drug and also lack of nutritional problem or enhancing factor of opioid effects. But these findings are incompatible to below studies.

Using MRI, cerebral cortex volume was reduced and ventricular size was increased secondary to toxic dosage of morphine. Perazos and Anroos (1998) finding showed that brain volume was reduced secondary to excessive use of heroin. Reduced brain and hypophysis volume due to chronic use of opioids has also been reported by Teoh *et al.* (2000). And finally, Teoh *et al.* (2000) showed that heroin administration to mice will result to neural necrosis. All these findings may be due to high dosage or long period of administration and In this study, omitted these two factors.

CONCLUSIONS

In conclusion, the present study indicates that the mean total brain mass, gray matter and white matter showed no significant changes.

It is believe that the dose and duration of buprenorphine and methadone in this study has no effects on brain grossly. So, probably use of buprenorphine and methadone for addiction treatment is safe at least on macroscopy. We suggest perform molecular and microscopic studies about effect of these drugs on brain and its component for demonstration of pathologies of these drugs on brain if changes are exist.

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