

Evaluation of Total Brain Acetylcholine in rats treated with inhaled Tetrahydrocannabinol. (A Bioassay Study)

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Abstract

A Bioassay study using rabbit ileum was used to evaluate changes in Total Brain Acetylcholine of wistar rats treated for 4, 8 and 12 weeks with inhaled tetrahydrocannabinol smoke; a widely abused substance worldwide. Puffing of cannabis smoke was performed with the use of a Hamilton syringe delivering 100-ml puffs at 20-sec intervals into the nose -only manifold for a total of 5 minutes. Smoke was first pumped into a 500ml dilution chamber with the aid of a vacuum pump. The smoke was then displaced from the dilution chamber through the nose-only manifold at 300 ml/min; that is the rats received Inhaled THC at 5ml/sec for 5 minutes. After treatment the rat Brains were accessed for Total Brain Acetylcholine (TBA) levels. To achieve this; an increasing amounts of homogenized Brain extracts from the rats in each group and Laboratory acetylcholine were used and their ability to contract the rabbit ileum when introduced into an organ bath was recorded as height of contraction which was then used to calculate actual TBA concentration. This was first done for all groups to first determine an effective dose to be used. For the actual Bioassay study the chosen effective dose of 0.5ml of Brain preparation from Control group, 4, 8, 12 weeks treated groups and standard Laboratory Acetylcholine were used to determine the concentrations of Total Brain Acetylcholine present in the Brain extracts. Atropine was also introduced to ascertain the presence of Ach in the Brain extracts. The experiments showed significant increase in TBA in the treated rats showing an accumulation of Ach in the Brain due to THC inhalation. This is part of a series of studies to establish the influence of THC on the neurotransmitter acetylcholine and cells of the Brain stem for its possible importance in neurotransmitter deficiency and related neurodegenerative diseases.

Keywords: Total Brain Acetylcholine (TBA), Tetrahydrocannabinol (THC), Inhalation treatment, Bioassay, Rabbit ileum, Height of Contraction, Nose-Only smoke model.

1. Introduction

There is growing evidence that tetrahydrocannabinol from cannabis is been used as remedies mostly as analgesics and appetite stimulant (Campbell et al., 2001; Pertwee, 2001; Iversen and Chapman, 2002). Among its other effects includes dilating bronchioles in the lungs and reducing intraocular (within the eye) pressure (Ameri et al., 1999). Other suggested uses are its use as possible therapy for glaucoma and asthma (Grilly et al, 2006). On the contrary so much is also been said on THC related disorders and cellular changes caused by its use (Abood and Martin, 1992).The present work evaluates the changes in the level of Total Brain acetylcholine in rats treated for 4, 8 and 12 weeks with inhaled THC through a nose – only smoke administration model (Sarafian et al, 2006). Neurotransmitter changes have been areas of investigations used to show drug action. Of the acetylcholine related hallucinogens, some enhance the neurotransmitter and some inhibit it (Charles, 2010). The presence of increased acetylcholine in the brain might be because cannabinoids inhibits the cholinesterase thereby blocking the uptake of acetylcholine by cholinergic neurons in the brain (Katona et al., 2001; Wilson and Nicoll, 2001). Our results will confirm THC's effect on Total Brain Acetylcholine and give a clue to its influence on the Brains which can give insights to its damaging effect or as remedies for illnesses.

2. Materials and Methods

2.1 THC (Cannabis)

Cannabis was collected from the National Drug Law Enforcement Agency (NDLEA) office Abuja; it was identified and authenticated by the National Drug Law Enforcement agency, Gudu, Abuja. Ethical approval was also received from the University of Jos ethics committee. The cannabis was burnt in a nose-only administration chamber and administered by 'Puffing' performed with the use of a Hamilton syringe delivering 100-ml puffs at 20-s intervals into the nose only manifold for a total of 5 minutes. Smoke is first pumped into a 500ml dilution chamber with the aid of a vacuum pump. Smoke is then displaced from the dilution chamber through the nose-only manifold at 300 ml/min. That is they received Inhaled THC at approximately 5ml/sec. (Sarafian et al, 2006). See Fig. 3 & 4

2.1.1 Instrumentation

1. Nose- only smoke model – THC administration (Fig 3 & 4)
2. Homogenizer – Brain Extract Preparation
3. PH meter - Brain Extract Preparation
4. Organ Bath and Physiograph – Ach Bioassay (Fig 5 & 6)

2.1.2 Animals

Male albino rats weighing 180 – 260g were employed. They had free access to food and water except at the time of the experiments. There were 4 groups with five rats per group.

2.2 Brain Extract Preparation for Total Brain Acetylcholine

Albino Rats after appropriate treatment with inhaled THC were sacrificed and their Brains were removed excluding the cerebellum. The Brains were homogenized in a solution of ice-cold thyroid and Eserine. It was then acidified using 0.5 M HCL to a PH of 3-4. The Homogenates were centrifuged at 3000 rev/min. The supernatant was removed and neutralized with 0.5 M NaOH. The extract was tagged and assayed for total brain acetylcholine (Milosevic, 1970; Ramesh, 2008).

2.2.1 Bio-assay Studies

Rabbit Ileum was used for the Bioassay. The Ileum was put in an isolated organ bath in thyroid solution and the contractions were recorded using a physiograph on a chart in mm. The contractions were recorded at a Speed of 0.5mm/s; Sensitivity of 500 microvolt's. The five (5) test solutions below were analyzed to ascertain the concentration of Total Brain Acetylcholine present in them using Laboratory acetylcholine as the standard.

- | | |
|--|---------------|
| 1. Laboratory acetylcholine (Standard) | - ACh |
| 2. Total Brain ACh from control | - TBE control |
| 3. Total Brain ACh from 4wk THC treated | -4wks THC |
| 4. Total Brain ACh from 8wk THC treated | -8wks THC |
| 5. Total Brain ACh from 12wk THC treated | -12wks THC |

For the Bioassay study the above were introduced into the thyroid solution with the rabbit Ileum in an organ bath and the height of contractions cause by the introduction of the solutions were recorded on a chart. The height of

contractions were measured and recorded in (mm) as the response of the Ileum to the substances introduced. (Ramesh , 2008). See Fig 5 & 6

Atropine was used as an acetylcholine antagonist to confirm the presence of acetylcholine in brain extract.

2.3 Statistical analysis

Data from all experiments were statistically evaluated using Student’s t test. The height of contraction was used to calculate actual concentration of Ach present in Brain Extracts.

3.0 Results

Four, eight and twelve weeks after inhalation treatment of 0.5ml/sec of THC from cannabis there was marked increase in the total brain acetylcholine content of the rats brains. The results summarized below; on table 1 shows that there was effective ileum contraction with 0.5ml of acetylcholine and of the 4 brain extracts. Also in table 2 which shows calculated concentrations there was significant increase in height of contraction and consequently the acetylcholine concentrations of the THC treated groups against the control group using the Student T-Test.

Atropine blocked the activities of the Brain extract and the laboratory acetylcholine (See Chart 6 & 7).

3.1 The determination of effective dose

From bioassay Charts 1, 2 and graphical Fig 1, effective dose of 0.5ml was chosen for the Bioassay analysis for Total Brain Acetylcholine in the Brains extracts of all groups and standard Acetylcholine as indicated on Table 1.

3.1.1 Determination of Acetylcholine concentrations

Charts 4 and 5 from bioassay study show the contraction of Rabbit ileum due to administration of the 4 Brain Extracts and standard acetylcholine. Change in height of contraction was recorded in mm and calculate in microgram (ug). See table 2.

Calculated value for concentration of acetylcholine content of Brain extract:

Calculating concentration of Ach in THC treated Brain using a method and formula by Ramesh Goyal in 2008

$$\text{Concentration of Unknown} = \frac{n_1}{t} \times \text{antilog} \left(\frac{T - S_1 \times \log \frac{n_2}{S_2 - S_1}}{n_1} \right) C_s$$

C_s = Concentration of standard Acetylcholine = 0.5ml of 10ug/ml = 5ug

Table 2 shows the summary of the means of calculated concentration of all Groups: (See Fig 2)

Concentration of standard Acetylcholine used was 0.5ml of 10ug/ml Ach. (5ug) microgram corresponds with the calculated mean of 5.045 +/- 0.5228.

*Atropine antagonist effect on acetylcholine and brain extract was confirmed on the bioassay charts 6 & 7.

4.0 Discussion & Conclusion

The present findings show that inhaled THC from cannabis caused significant increase in the level of Total Brain Acetylcholine in the tests considered. A comparative study among the groups indicated that the difference was statistically significant (at $P < 0.05$) for the mean Total Brain acetylcholine of THC treated groups against the control. The 8 weeks and 12 weeks treated groups were highly significant (at $P < 0.01$) when compared with the control though there was no significant difference between the 8 weeks and the 12 weeks. The calculated concentration of Total Brain acetylcholine showed that THC increased Total Brain acetylcholine in the 4 weeks, 8 weeks and 12 weeks. All tests involved a Bioassay of Total Brain acetylcholine using a rabbit ileum to detect changes in acetylcholine concentration in the brains of THC treated rats. ACh content of the Brain increased due to administration of inhaled THC which in turn will alter the neurotransmitter level in the brain. The total brain Acetylcholine content could be affected by drugs or substances crossing the Blood Brain Barrier (Milosevic, 1970). Acetylcholine (ACh) is a neurotransmitter chemical of the CNS and peripheral nervous system and its increased secretion can be attributed to increase psycho activity in the Brain after THC administration. Of the acetylcholine related hallucinogens, some enhance the neurotransmitter and some inhibit it (Charles, 2010). These results show that THC enhances the neurotransmitter Acetylcholine and the study encourages further study on the use of THC as remedies for acetylcholine related brain disorders and deficiency. It should also be noted that the presence of increased acetylcholine in the brain might be because cannabinoids inhibits the cholinesterase thereby blocking the uptake of acetylcholine by cholinergic neurons in the brain (Katona et al., 2001; Wilson and Nicoll, 2001)

References

1. Abood, M., and Martin, B. (1992). Neurobiology of marijuana abuse. Trends in Pharmacological Sciences, 13,201-206.
2. Ameri, Angela (1999). The effects of cannabinoids on the brain. Progress in Neurobiology, 58, 315-348.
3. Campbell FA, Tramer MR, Carroll D, et al. Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. [Review]. *BMJ* 2001; 323: 13–6.
4. Chait, L.D., and Burke, K.A.(1994). Preference for high-versus low-potency marijuana. Pharmacology, Biochemistry, 49,643-647.
5. Charles F Levinthel, (2010). Drug, behavior, and modern Society; 6th Edition, 167 -188.
6. Grilly, David M. (2006). Drugs and human behavior (5th ed.) Boston: Allyn and Bacon, p. 268.
7. Iversen LL, Chapman V. Cannabinoids: a real prospect for pain relief? [Review]. *Curr Opin Pharmacol* 2002; 2: 50–5.
8. Katona I, Rancz EA, Acsády L, et al. Distribution of CB₁ cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci* 2001; 21: 9506–18.
9. M. P. Milosevic - Institute of Pharmacology and Toxicology, Medical Faculty, Belgrade, Yugoslavia; Acetylcholine content in the brain of rats treated with paraoxon and obidoxime, *Br. J. Pharmac.* (1970), 39, 732-737.
10. Pertwee RG. Cannabinoid receptors and pain. [Review]. *Prog Neurobiol* 2001; 63: 569–611.
11. Ramesh K. Goyal; 2008, L. M. College of Pharmacy, Navrangpura, Ahmedabad- 380 009 (18.01.2008): Pharmacology : Principles and methods of Bioassay. 5 – 11, 14, 34.
12. Sarafian Theodore A, Nancy Habib, Michael Oldham, Navindra Seeram, Ru-Po Lee, Laura Lin, Donald P. Tashkin, and Michael D. Roth. Inhaled marijuana smoke disrupts mitochondrial energetics in pulmonary epithelial

cells in vivo: Am J Physiol Lung Cell Mol Physiol 290: L1202-L1209, 2006.
 doi:10.1152/ajplung.00371.2005 ,1040-0605/06.

13. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 2001; 410: 588–92.

Table1: The contraction of rabbit ileum in the determination of Effective quantity in ml (The specimens were administered in increasing order from 1-6) See charts 1, 2 & Fig 1.

| S/No. | 1 | 2 | 3 | 4 | 5 | 6 |
|--|------|-----|------|-----|------|-----|
| Doses administered in ml. | 0.05 | 0.1 | 0.15 | 0.2 | 0.25 | 0.5 |
| Height of contraction in mm for ACh | 10 | 10 | 10 | 13 | 13 | 16 |
| Height of contraction for control (mm) | 10 | 11 | 11 | 12 | 12 | 12 |
| Height of contraction for THC (mm) | 10 | 11 | 11 | 15 | 16 | 16 |

(Normal contraction of rabbit ileum was – 10mm)

Fig 1: Graphical representation for the determination of effective dose

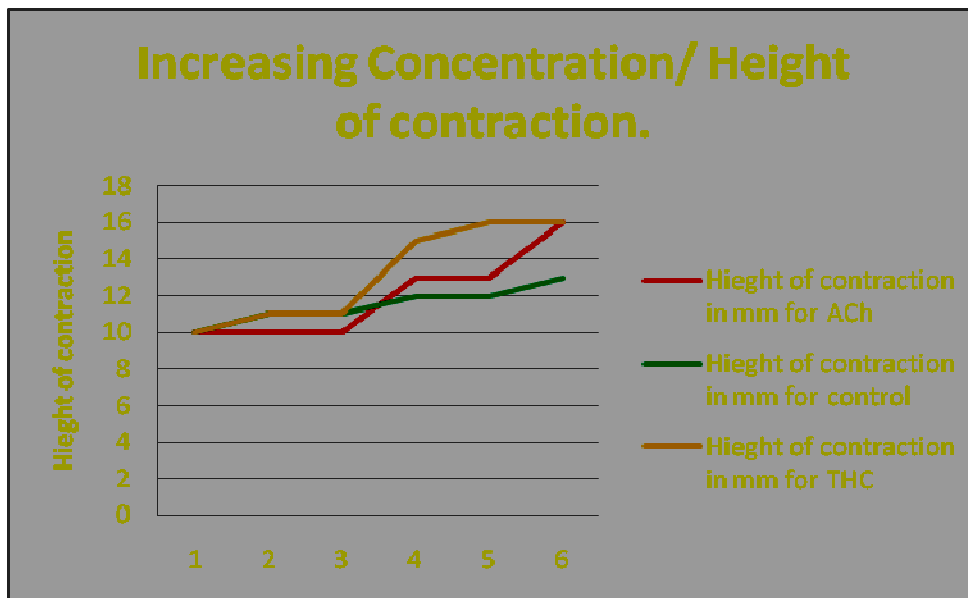


Table 2: Summary of the calculated concentration of all Groups: (See Fig 2)

| Group | Height of Contraction (mm) | Calculated Concentration (ug) |
|---------------------------|----------------------------|-------------------------------|
| 1. Standard Acetylcholine | 15.60 +/- 0.2449 | 5.0450 +/- 0.5228 |
| 2. Control Group | 11.00 +/- 0.3162 | 0.8458 +/- 0.1620 |
| 3. THC treated (4 wks) | 14.80 +/- 0.3742 | 5.5658 +/- 1.0646 |
| 4. THC treated (8 wks) | 16.00 +/- 0.3162 | 9.7060 +/- 1.2690 |
| 5. THC treated (12 wks) | 16.20 +/- 0.3742 | 10.183 +/- 2.0271 |

Concentration of standard Acetylcholine used was 0.5ml of 10ug/ml Ach. (5ug) microgram corresponds with the calculated mean of 5.045 +/- 0.5228.

Fig 2 shows a summary of the calculated concentration of acetylcholine for all groups and the standard acetylcholine used.

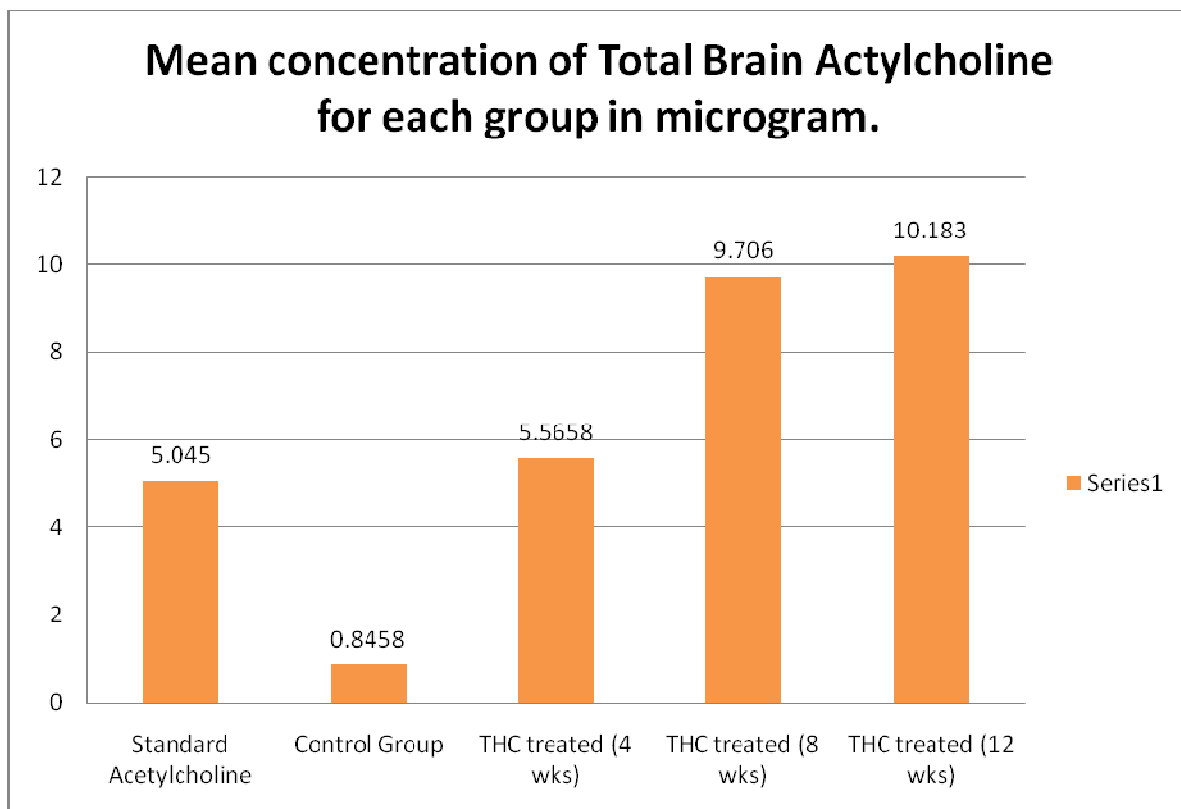


Chart 1 & 2: Shows determination of Effective dose

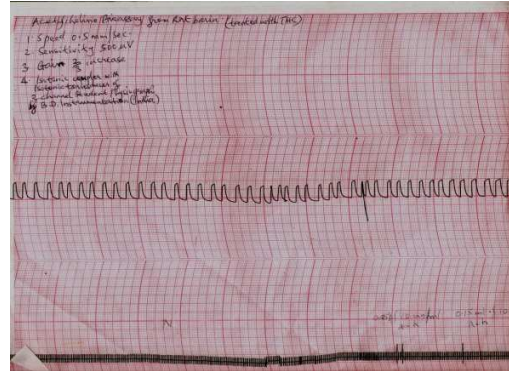
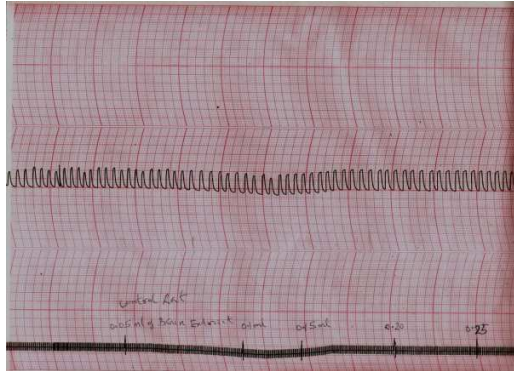


Chart 4 & 5: Shows the contraction of Rabbit ileum due to administration of Brain Extracts and acetylcholine (Bioassay Study)

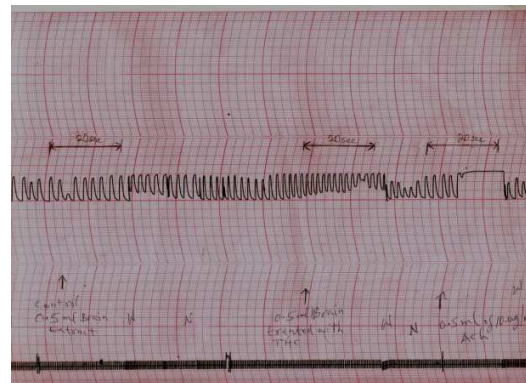


Chart 6 & 7 Shows atropine antagonist effect on acetylcholine and brain extract.

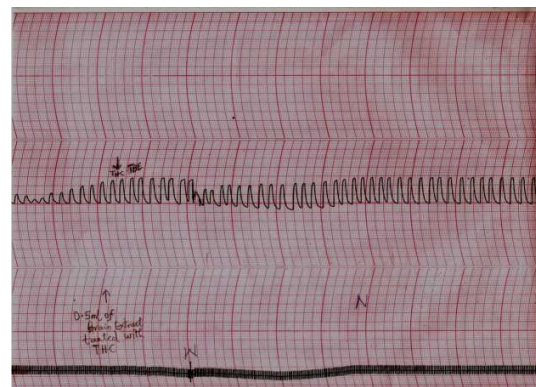


Fig 3 & 4 : NOSE-ONLY SMOKE EXPOSURE MODEL



Fig 5 & 6 : BIO-ASSAY STUDIES

