**Original article:**

# Effect of atropine on cardiac rhythm of Frog's heart- archived information

**[1]Dr. Rama Rao Bagayath, [2]Jyothinath Kothapalli**

[1]Professor, Department of physiology, MNR medical college& hospital, Sangareddy, Medak, Telangana
[2]Assistant professor, Department of Anatomy, MNR medical college& hospital, Sangareddy, Medak, Telangana
Corresponding author: Dr. Rama Rao Bagayath
Date of submission: 11 May 2014; Date of Publication: 15 September 2014

**Abstract:**
It is well accepted fact that atropine is a parasympatholytic drug and competes with Ach for muscarinic receptors. Hence, its use in bradycardia with or without hypotension has been suggested by some workers. But others opposed it because of unwanted tachycardia may result as consequence of inaccurate dose adjustment. In view of existing controversy we thought it proper to explore this field. For the present study we selected frogs (Rana Tigrina) of both sexes, weighing=475gms. The heart was exposed, isolated and perfused with ringer's solution, using Syme's cannula at a constant pressure and perfusion rate. After recording normal cardiogram 0.5ml of Ach (1:10000) was infused to induce experimental bradycardia and effect was recorded on kymograph. After recover of heart the same dose of Ach was infused followed by atropine infusion (0.1 mg/kg body weight) and effects were again recorded. It is observed that bradycardia decreased and amplitude increased and no tachycardia recorded. The clinical correlates and statistical significance will be discussed.
**Keywords:** Cardiac rhythm, Bradycardia, Atropine, Acetylcholine

**Introduction:**

It is suggested that excessive vagal discharge manifested by Bradyarthymias is an important precursor of ventricular fibrillation and an important factor in the early high mortality from myocardial infarction. It has also been suggested that the damaged heart is more susceptible to vagal over activity (1, 2).

Reduction in cardiac output may lead to the progression of ischemia causing further extension of initially affected area. There is also experimental evidence that under such conditions early ectopic beats are more frequent and that the fibrillation threshold is significantly lowered (3).Keeping in view the above mentioned facts some workers suggested that atropine may be administered in small doses under careful monitoring system.(4) since the excessive vagolytic effect may precipitate ventricular ectopic rhythm which requires lignocaine for their control. Furthermore tachycardia complicating acute myocardial infarctions is known to be as hazardous as bradycardia. (4)In acute myocardial infarction the administration of morphine sulphate for pain may further decrease the heart rate. (5) Atropine has been widely suggested as the treatment of choice both for the low output and for ventricular extra systoles


www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

associated with bradycardia of AMI (4, 6, 7, 8, 9, 10 and 11).

However the appearance of ventricular fibrillation in two patients and short bursts of repetitive ventricular firing in a third patient after intravenous administration of atropine in the space of three months indicates that the use of this drug may not be completely without risk.These controversial reports stimulated us to take the present study to find out effect of atropine on experimentally induces bradycardia, before we could say that its use is beneficial or harmful in clinical conditions like bradycardia with or without hypotension and acute myocardial infarction and bradycardia.

**Materials and methods:**

**Isolated frog's heart preparation:**

Frog was pitched following a routine technique, abdomen and thorax were exposed and pericardium was removed. A thread was passed behind the sinus venosus and a loose knot was applied. Syme's cannula was introduces in to the sinus venosus after making a v-shape slit. The knot was tightened and the fluid was allowed to pass through the chambers. After this a heart was separated carefully and aorta was cut. The apex of the heart was hooked to a lever, which was adjusted on the moving drum as shown in the (fig.1). Following precaution was also observed before the experiment.

1. Frog's ringer solution was used throughout the experiment.
2. Room temperature was noted
3. PH of ringer solution was also determined
4. The pressure was maintained in the cannula by keeping the fluids at the same level in the Syme's cannula and in the reservoir where the red line was marked so that all the experiments could be conducted at the same pressure. Twenty six big size frogs weighing mean 350gms of both sexes belonging to family of Rana pentadactyle were used throughout the experiments.

Isolated frogs heart as described above was perfused with ringer solution. When the height of contraction and rhythm were regularized. A sample of control graph was recorded and then 0.5cc of 1:10000 acetylcholine solution was injected in to Syme's cannula. The effect of the ach was recorded and waited until the effect of ach was warded off. After recovery of the heart same dose of ach was infused followed by atropine infusion (0.1 mg/kg body weight) and amount infused was 0.5 cc and effects were again recorded. Then the infusion process was reversed i.e. atropine infusion was immediately followed by ach administration and their effect was also recorded. Just for the sake of comparison few experiments() were also carried out on heart in situ, adopting the fore mentioned procedure stepwise and also reverse step for recording effects on rhythm and force of infused drugs.

The data prepared in the form of tabled and percentage changes for the individual experiment were calculated and means for all the steps were also calculated and compared with normal rhythm to interfere the effect of infusion of given drug.

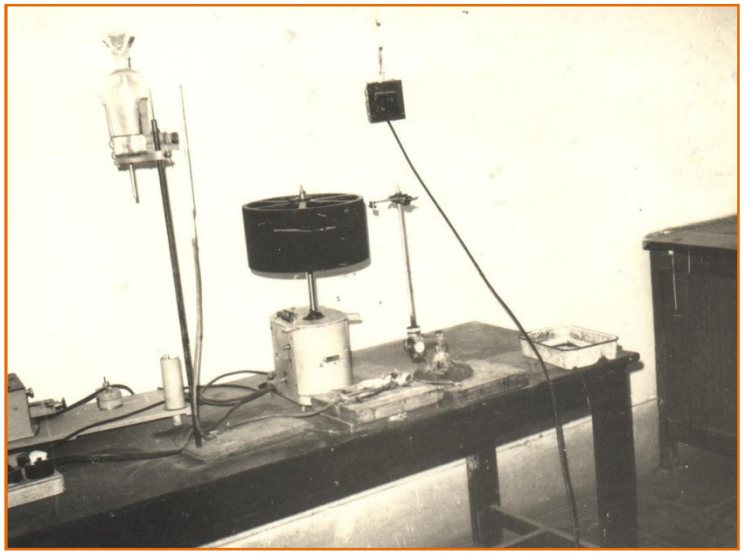

**Fig.1. experimental set up for the perfusion of frog's heart in situ**

**Results:**

As mentioned under material and methods on each exposed and isolated frogs heart three sets of experiments are carried out giving sufficient time for complete recovery from previous experimental set up. These are as follows-

1. Induction of experimental bradycardia by ach infusion (1:10000) to stimulate the clinical condition of bradycardia also to know the exact dose required for induction of bradycardia. The same dose is being used for subsequent experimental setup.
2. Effect of atropine infusion on experimentally induced bradycardia. This is to know as to whether atropine infusion attenuates experimental bradycardia or not. If so, how for it would be effective.
3. For third set up reverse procedure is adopted i.e. in the presence of atropine perfusion the ach is infused this is just for comparison with results of 2nd set of experiment mentioned.

| S.no | NCG/min | 1:10000 Ach 0.5 ml | Reduction % | Amplitude | 0.5ml Ach/ followed by atropine (dose 1mg/kg body wt) | Reduction % | Amplitude | Atropine followed by Ach (dose 0.25ml followed by 0.5ml) | Decrease % |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 23 | 20 | 13.04 | ↓ | 20 | 13.03 | ↑ | 22 | 4.34 |
| **2** | 34 | 29 | 14.70 | ↓ | 28 | 17.64 | ↑ | 29 | 14.70 |
| **3** | 34 | 31 | 8.82 | ↓ | 30 | 11.76 | ↑ | 30 | 11.76 |
| **4** | 34 | 18 | 45.45 | ↓ | 31 | 6.06 | ↑ | 30 | 9.09 |
| **5** | 33 | 27 | 48.18 | ↓ | 31 | 6.06 | ↑ | 29 | 12.12 |
| **6** | 33 | 20 | 39.39 | ↓ | 30 | 9.09 | ↑ | 30 | 9.09 |
| **7** | 33 | 25 | 24.24 | ↓ | 30 | 9.09 | ↑ | 30 | 9.09 |
| **8** | 36 | 15 | 58.33 | ↓ | 30 | 16.66 | ↑ | 26 | 27.77 |
| **9** | 32 | 21 | 34.37 | ↓ | 30 | 6.25 | ↑ | 29 | 9.37 |
| **10** | 34 | 19 | 44.11 | ↓ | 24 | 29.41 | ↑ | 29 | 14.70 |
| **11** | 37 | 25 | 32.43 | ↓ | 35 | 5.40 | ↑ | 37 | 0 |
| **12** | 27 | 20 | 25.92 | ↓ | 20 | 25.92 | ↑ | 20 | 25.92 |
| **13** | 20 | 19 | 5 | ↓ | 19 | 5 | ↑ | 19 | 5 |
| **14** | 30 | - | 100 | ↓ | 28 | 6.66 | ↑ | 28 | 6.66 |
| **15** | 32 | - | 100 | ↓ | 28 | 12.5 | ↑ | 28 | 12.5 |
| **16** | 28 | 23 | 17.85 | ↓ | 24 | 14.28 | ↑ | 24 | 14.28 |
| **17** | 33 | - | 100 | ↓ | 26 | 21.21 | ↑ | 26 | 21.21 |
| **18** | 26 | 20 | 23.8 | ↓ | 21 | 19.23 | ↑ | 15 | 42.30 |
| **19** | 31 | - | 100 | ↓ | 27 | 12.90 | ↑ | 28 | 9.67 |
| **20** | 30 | 6 | 80 | ↓ | 27 | 10 | c | 26 | 13.33 |
| **Mean** | **31** | **20/ min** | **35.14%** | | **26.95/min = 27/min** | **12.62%** | | **26.75/mn =27/min** | **13.64%** |



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

The results of three experimental setups are tabulated as shown in table1. It stands clearly the individual variations in heat rate, ranging 20-30/minute; the mean resting isolated heart rate is 31/minute. The ach infused bradycardia also exhibits individual variations ranging 6-31/minute. However the mean heart rate ach induced bradycardia is 20/minute. The percentage fall after ach infusion varies between 8-100% and mean is 35.14% when compared with normal this reduction in rate (i.e. negative chronotropic effect) is always associated with negative inotropic effect of ach (fig. no. 3)

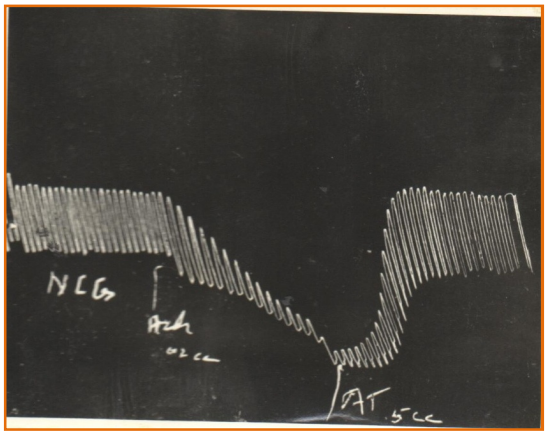

**Fig. no. 2: Effect of acetylcholine followed by atropine in vitro**

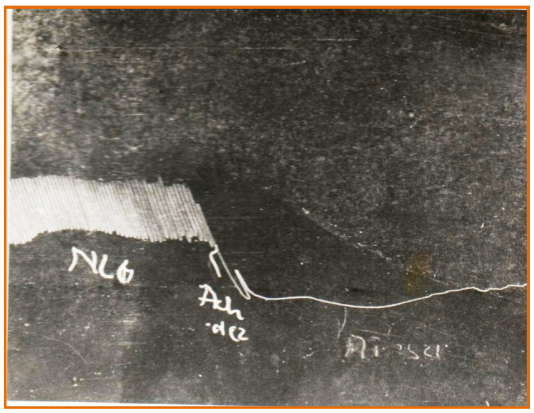

**Fig. no. 3: shows effect of acetylcholine (1:10,000)**

Table 1 also elicits the atropine infusion effect on bradycardia i.e. the experimentally induced bradycardia by ach infusion could be overcome successfully by successive infusion of atropine (1 mg/kg body weight). It shows individual variations in the heart rate ranging 20-30/minute but mean heart rate is 27/minute. Percent fall in this setup i.e. ach infusion followed by atropine is ranging from 5-21% but mean percent of fall is 12.62% i.e. certainly less than the effect of ach alone which is equal to 35.14%. In other words bradycardia in reduced by atropine and at the same time force is also greater compared to normal (fig. no. 3) i.e. +ve inotropic effect is also observed along with improvement of bradycardia.

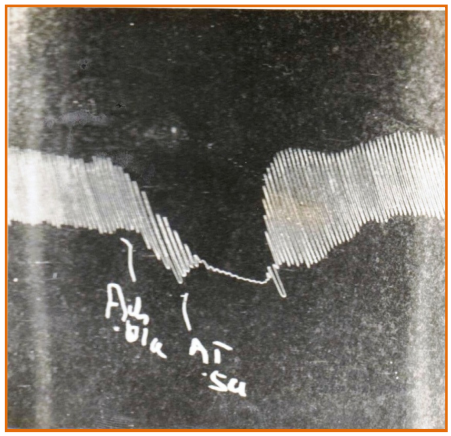

**Fig. no. 4 shows effect of acetylcholine followed by atropine in vivo**

On reversing the procedure for 3rd experimental set (i.e. atropine infusion followed by ach) using the same dose as in 2nd experimental setup there is not much differences. As the heart rate ranges from 15-37 and mean heart rate 26.75/minute (i.e.27/ minute) and percentage fall in heart rate compared to normal is also ranged from 0-42%. However, mean percentage fall is 13.64% i.e. almost same as in 2nd experimental set i.e. ach infusion followed by atropine which is equal to 12.62%. There is hardly a difference of 1 %.



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

| S.NO | NCG/ min | 1:10000 Ach 0.5 ml | Reduction % | Amplitude | 0.5ml Ach/ followed by atropine (dose 1mg/kg body wt) | Reduction % | Amplitude | Atropine followed by Ach (dose 0.25ml followed by 0.5ml) | Decrease % |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 28 | 0 | 100 | ↓ | 29 | +3.57 | ↑ | 24 | 14.28 |
| **2** | 28 | 23 | 17.85 | ↓ | 28 | 0 | ↑ | 24 | 14.28 |
| **3** | 18 | 15 | 16.66 | ↓ | 18 | 0 | ↑ | 15 | 16.66 |
| **4** | 22 | 10 | 54.55 | ↓ | 18 | -18.18 | ↑ | 18 | 18.18 |
| **5** | 20 | 15 | 25 | ↓ | 18 | -17.85 | ↑ | 19 | 5 |
| **6** | 28 | 13 | 53.5 | ↓ | 23 | -17.85 | ↑ | 25 | 10.7 |
| **Mean** | **24/min** | **12.66/min** | **43.93%** | | **22.33/min** | **9.57%** | | **20.83/min** | **13.18%** |

Table 2 shows the result of experiments carried out on heart in situ following the same three experimental set ups as for mentioned for isolated heart. The 1st column shows the normal heart rate the 1st column shows the normal heart rate ranging 18-28/minute. Mean heart rate 24/minute on induction of ach infuse bradycardia it ranges between 0-13/minutes mean heart rate is 12.66/minute. Therefore percentage reduction in heart rate ranges 16.66-100% and means percentage fall is 43.93%. It is associated with as before –ve inotropic effects. When ach infusion followed by atropine the changes induced in rhythm is ranged between 18 to 29/minute, mean heart rate is 22.3/minute and percentage fall ranges 0-17.85%, mean percentage is 9.57% associated with +ve inotropic effect i.e. atropine has restored the rhythm nearly normal inspite Ach infusion in other words as before in this also reduction in bradycardia is associated with better myocardial performance as indicated by +ve inotropic effect on reverse perfusion (i.e. atropine infusion followed by Ach). The bradycardia ranges between 15-25 beats/minutes; mean heart rate is 20.83/minute. The percentage falls in heart rate is 10.7% to 18.18% and mean percentage fall is 13.18%. When comparison of percentage falls in heart rate in situ and isolated there are little differences.

**Discussion:**

Acetylcholine is a well known chemical mediator of all cholinergic chemical nerve fibers, hence its role as chemical transmitter of cholinergic impulses has been established since long (12).Atropine is also a well known peripheral parasympatholytic blocking agent. When it more or less completely blocks the vagus nerve cardio vascular effects rather constant.



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

Recently its effects on cardiac rate, rhythm and conduction have been subjected to conflicting interpretation by number of investigators (13).The influence of heart rate on incidence of arrhythmias during AMI has been a topic of considerable controversy and speculations. According to Dr. Stephen E. Epstein (chief cardiologist in U.S.A) of the 600000 people who die annually in United States for AMI are approximately 2/3rds die before receiving medical aid. (14, 15, 16) according to other groups 50% of patients experiencing an AMI die before receiving medical aid. (17, 18)

Recently it has been suggested that bradycardia may be one of the important causes of electrical instability leading to ventricular fibrillation in the pre-hospital phase. The implication of these observations seems straight forward bradycardia is a potentially dangerous rhythm frequently encountered in pre-hospital phase of AMI. With the advent of mobile CCU it seemed reasonable for atropine treatment of bradycardia occurring before hospitalization (4).

More recently the potential impact of use of atropine in this situation was enormously increased by the suggestion that many patients dying in the pre-hospital phase while awaiting medical aid (19). This approach appeared especially attractive because it is commonly believed that a moderate increase in rate under such circumstances rarely has any deleterious effects (20). However use of atropine in such condition challenged by others.

Keeping in view fore mentioned facts we thought instead of taking up AMI and atropine use straight under these controversial preposition. It is better first to explore its effects on experimental bradycardia model. Hence, as mentioned under methods and results experimental bradycardia could be induced successfully. Its consistency and reproductability tested number of times both in isolated and intact frog's heart. In few cases the same dose produced 100% inhibition. These effects can be explained on the basis of muscarinic action of Ach. 100% effect of same dose might be due to small heart. Then vagolytic action of atropine is tested. It is interesting to note that it could reduce the experimentally induced bradycardia even the 100% effect of Ach (shown in table 1& 2). It is worth mentioning here not only that is observed it also induced reversal of negative inotropic and negative chronotropic effects of Ach on successive infusion of atropine i.e. atropine increased force of contraction of myocardium and reduced bradycardia. However tachycardia never observed neither in isolated heart nor in heart in situ (). Of course they adopted reverse infusion technique i.e. on atropinized heart the Ach effects tested, so we also reverse the technique i.e. atropine infusion followed by Ach (table 1& 2). Even then the difference is hardly 1% i.e. less bradycardia in latter situation because of priority of atropine action. Fore mentioned authors also added extra in perfusate ca probably i.e. responsible for tachycardia and differences in rate response.The same dose of Ach and atropine combination tried successfully several times. Of course giving sufficient time for complete recovery (5 to 15 minutes) in between the two trials. With this precaution we could repeat the experimental set up. However, we could not record the tachycardia or fibrillation or premature beats as reported by others (21, 22).

**Conclusion:**

In short atropine use in case of experimental bradycardia is beneficial in both conditions (i.e.



www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

isolated and in situ heart). There is no danger of unwanted tachycardia during selected experimental study. However we would like to suggest its effects in AMI with bradycardia in experimental set up should be given as a trial before its use in clinical cases and also dose and response to be recorded to have a benefit than endangering life. . For the present study we selected frogs (Rana Tigrina) of both sexes, weighing average is 475gms. The heart was exposed, isolated and perfused with ringer's solution, using Syme's cannula at a constant pressure and perfusion rate. After recording normal cardiogram 0.5ml of Ach (1:10000) was infused to induce experimental bradycardia and effect was recorded on kymograph. After recover of heart the same dose of Ach was infused followed by atropine infusion (0.1 mg/kg body weight) and effects were again recorded. It is observed that bradycardia decreased and amplitude increased and no tachycardia recorded. The result of three experimental setups stands clearly the individual variations in heat rate, ranging 20-30/minute; the mean resting isolated heart rate is 31/minute. The ach infused bradycardia also exhibits individual variations ranging 6-31/minute. However the mean heart rate ach induced bradycardia is 20/minute. The percentage fall after ach infusion varies between 8-100% and mean is 35.14% when compared with normal this reduction in rate (i.e. negative chronotropic effect) is always associated with negative inotropic effect of other.

**References:**


1. Brawn .L and sammet .B (1928) Dt.Arch, clin.med. 161; 257
2. Narhanson, M.H (1946) ibid 77; 491.
3. Han .J, millet .D et al (1966) Am. Heart. j 71; 481.
4. Adgey A.A.J, gedder, J.S. Mulholland, H.C. et al 1968 lancet 2;1097.
5. Thomas M, malmerona, R. filllmore et al (1965) Br. Heart. J. 27; 875.
6. Thomas M and woodgate D, (1966) Br. Heart. J. 28; 409.
7. Shillingford J and Thomas M (1968) Am. Heart. j 75; 843.
8. Lown B et al (1967) JAMA 199; 188.
9. Lown B et al (1969) Am. J. med, 46; 705.
10. Kimball J.T and killip T (1968) frog. Cardiovasc.dis, 10;483
11. White B.B (1971) in therapy in acute coronary care, year book of medical publishers, pp.34.
12. Mintz (1955) in "the role of humorall agents in nervous system'' by Thomas C.C, 31 Newyork.
13. Averill K.H, lamb L.E (1959) Am. J. Med. Sci. 237;304
14. Kuller .L, Lilienfeld .A et al (1966) circulation 34;1056
15. Weinblatt .E, Shapiro .S, frank C.W et al (1968) Am. J. public health 58;1329
16. Mc neilly R.H and pemberton .J (1968) Br. Med. J. 3;139
17. Banton C.R and Peterson D.R (1963) new eng. j. med. 268;569
18. Kuller .L (1969) Am. J. cardio. 24;617
19. Sarnoff S.J. (1970) wiggers award lecture. Am. Physiological society, Atlantic City N.J.
20. Kumar .R, Joison .J et al (1971) J. clin. Invest. 50;217




www.ijbamr.com P ISSN: 2250-284X , E ISSN : 2250-2858

21. Lunde .P (1976) Acts. Med. Scand 199;369
22. Margenson .L and Orinius .E. (1971) acta. Med. Scand. 190;495