

CHAPTER II

MATERIALS AND METHODS

A. Animals

Male and female albino rats weighing 250-350 gm and fed ad libitum were used throughout this study

B. Preparations of isolated rat atria

Rats were sacrificed by blowing on the head. The chest was quickly opened and the heart was removed. The excised heart was immersed in oxygen-saturated Locke solution of the following composition: 155.8 mM NaCl, 5.6 mM KCl, 2.15 mM CaCl₂, 1.8 mM NaHCO₃ and 5.0 mM glucose. The ventricle and other extraneous tissues were carefully removed, the remaining atrium was then transferred and mounted in the 25 ml isolated organ-tissue bath chamber (Phipps & Bird, Inc.) containing Locke solution aerated with 100 % oxygen. The temperature of the bath was maintained constant at 37°C. A preload of 1 gm was applied to the atrium in every experiment performed. The rate and contractile force was recorded with isometric force transducer connected to a recorder (Bioscience). The atrium was allowed to equilibrate until stable rate and contractile force was observed before the experiments began.

C. Electrical stimulation of the atria

Electrical stimulation of the atria was achieved by field stimulation with platinum electrode. The stimulus strength was 5 V and the duration was 5 m sec. The frequency of stimulation varied according to experimental conditions; with the left atria, however, the frequency was kept constant at 250/min.

D. Preparation of methyl capsaicin

Methyl capsaicin was prepared from capsaicin by the method described by Lapwort and Royle (1919) as follows:

500 mg capsaicin was dissolved in 20 ml absolute methanol. This solution was then placed in an ice-bath, and 0.5 ml of dimethyl sulfate was added. To this mixture, 1.5 ml of 1.25 M sodium hydroxide was slowly added drop by drop with frequent stirring during the one-half hour period. When all the sodium hydroxide had been added, the reaction mixture was further stirred for half an hour. The solution was then evaporated to small volume and ice-cooled to precipitate methyl capsaicin. This compound was purified by multiple crystallizations from dilute methanol and dried at room temperature.

E. Reagents and drugs

Capsaicin and carbachol were purchased from Sigma Chemical Co. Norepinephrine and isoproterenol were obtained from Stering Drug Inc. Propanolol was obtained from I.C.I; atropine sulfate and epinephrine were obtained from the Government Pharmaceutical Organization.

Capsaicin and methyl capsaicin were dissolved in absolute ethanol, all other drugs were dissolved in either distilled or sterile water.