"In Vitro" Measurements of Spontaneous Smooth Muscle Contractions: A Screening Method for *"In Vivo"* Exposure

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The purpose of this investigation was to determine the usefulness of a neuromuscular screening method involving "in vitro" measurements of smooth muscle contractions for "in vivo" exposure to environmental pollutants. This procedure would then assist in defining the threshold levels of effect and possible mechanisms of action of the pollutants.

The physiological and histological measurements of smooth muscle have emphasized the extreme variability of this tissue. However, there does exist some similarity in the contractile process for smooth muscle in various organs (BOZLER, 1962). Thus it was necessary to select a parameter of contraction whose "in vitro" measurements would be characteristic for that given smooth muscle preparation. Such a selection would not require each smooth muscle preparation to serve as its own control. The rate of contraction frequency was selected as the parameter. Measurements were obtained by means of the interval histogram pattern (IHP) for the "in vitro" spontaneous contractions of the duodenal smooth muscle.

To establish the general applicability of this method as a model for screening of neuromuscular activity, the effects of sex, age, pH, Ca^{++} concentration, temperature and circadian rhythm were assessed.

Procedure

White rats were sacrificed by cervical dislocation. Ties were made approximately two-centimeters apart on the duodenum immediately distal to the pylorus. The excised tissue was placed in modified Ringer's solution (pH 7.5) (SANTOLUCITO and WHITCOMB, 1971) and allowed to equilibrate for 1 hour at 7°C prior to recording. A 7 mM/L concentration of HEPES was used. The tissue was mounted for isometric recording in 100 mls of solution, aerated with compressed air and maintained at 36°C. A fifteen minute equilibration period was allowed between mounting and recording of the IHP. Five minute equilibration period was allowed following the change to a solution of a different pH.

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The contractions were monitored by a Statham Strain guage and recorded on electromagnetic tape and dynograph paper. The general procedure was to record 500 contractions at pH 7.5, then replace the pH 7.5 solution with a pH 6.5 solution and record an additional 500 contractions. Finally the pH 6.5 solution was replaced with pH 7.5 solution (pH 7.5R) for a final recording of 500 contractions. The interval histogram analysis was done by a Nuclear Chicago Data Retrieval Computer Model 7100. An IHP was done for each phase of a given experiment. After the selected number of preparations had been recorded, an IHP was made which represented the total response. Most of the figures represent the summation of four animals thus 2,000 spontaneous smooth muscle contractions.

Results

This data has been presented using interval histograms rather than average rates. In Figure 1 are three IHPs each representing the frequency response of 500 contractions. In addition the average rates are listed. These were based on consecutive 5 minute counting periods during the recording of the 500 contractions. Since the IHP gives a frequency distribution it was assumed that this was a more accurate representation of the spontaneous contractions.

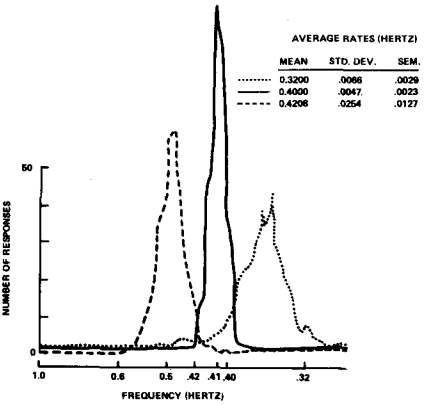

Figure 1

The IHP of the smooth muscle was defined in terms of sex, age, pH, temperature, circadian rhythm, and Ca^{++} concentration. Then the effects of selected pesticides were measured.

Sex:

Weanlings. Figures 2 and 3 are the IHP of four male and four female weanlings for pH 7.5, pH 6.5 and pH 7.5R. The IHP's overlap at comparable pHs for the male and female. However, at pH 7.5 the female's response was more diffuse than the male but less at pH 6.5.

Adults. Figures 4 and 5 are the IHPs of four male and four female adults. The degree of IHP overlap at comparable pHs is less than observed with the weanling. Also, the adult male has a slower rate of contraction than the adult female at all pHs. This is depicted in Figure 6.

Age:

Male. The slower frequency response for adult male as compared to the adult female was also evident when the adult male was compared to the weanling male (Figure 7). It would appear that with aging of the male, the mechanism regulating the spontaneous contractions of the smooth muscle was altered.

Female. The IHPs for the weanling and adult female rats were comparable. Thus aging in the female did not alter the rate of spontaneous contractions.

pH:

As depicted in Figures 2, 3, 4, and 5, the rate of spontaneous contraction was altered when the pH was reduced from pH 7.5 to pH 6.5. Whatever the mechanism of action, it was a reversible process as shown by the recovery at pH 7.5R.

Temperature:

The rate of spontaneous contraction was responsive to temperature. As shown in Fig. 8 for two male weanlings, increasing the temperature resulted in a faster rate of contractions.

Circadian Rhythm:

Since the rat's activity peaks in darkness, it was of interest to determine if the rate of spontaneous contractions reflected noctural and diurnal activity differences. Figure 9 is a comparison of four female adults sacrificed during daylight hours and four female adults sacrificed 3 to 4 hours following onset of darkness. The IHPs would suggest that the rate of spontaneous contraction is not affected by the animals circadian rhythm.

Ca^{++} Concentration:

In Ca^{++} free modified Ringer's pH 7.5, the degree of random responses increased. Thus the IHP was similar to pH 6.5 IHP in Figures 2, 3. 4, and 5. Upon the addition of 1 mM of EGTA to the Ca^{++} free solution, spontaneous contractions were blocked. Upon washing the blocked preparation with Ca^{++} containing modified Ringer's, the spontaneous contractions resumed. Increasing the Ca^{++} concentration from 2.5 mM to 20 mM/L at pH 7.5 did not alter the IHP. However, at 24 mM/L spontaneous contractions were blocked. On the assumption that the pH 6.5 effect was related to the Ca^{++}, increased concentrations of Ca^{++} were used at pH 6.5. The increased Ca^{++} concentration at pH 6.5 did not reverse the slower rate of contraction and greater degree of random response.

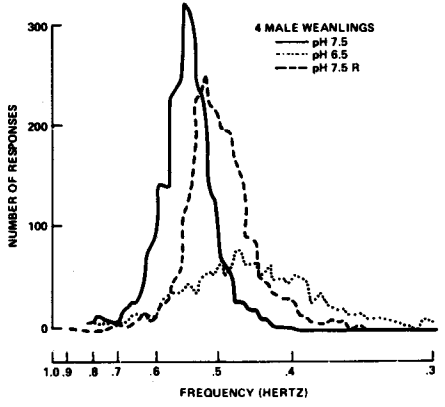

Fig. 2

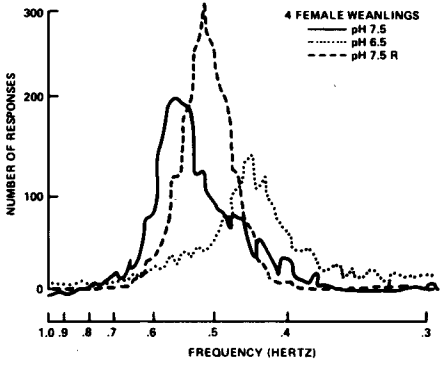

Fig. 3

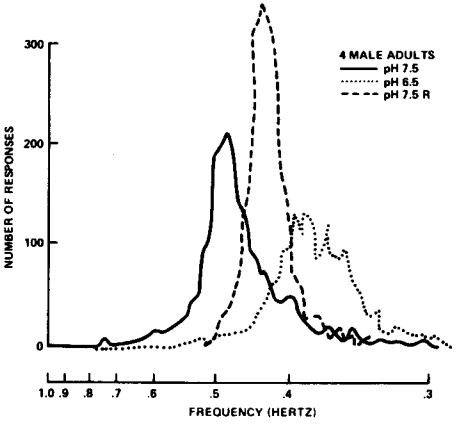

Fig. 4

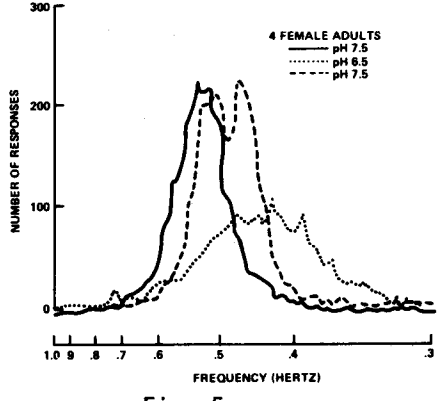

Fig. 5

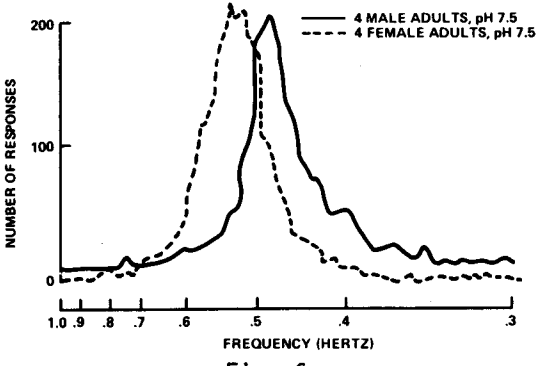

Fig. 6

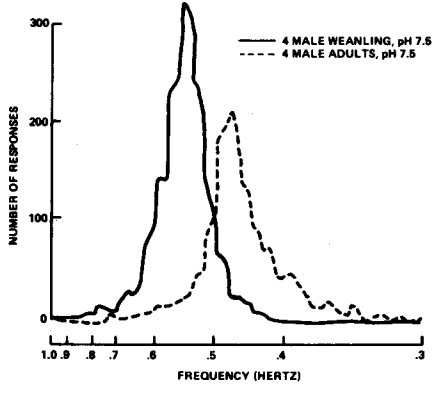

Fig. 7

Tissue Viability:

In Figures 2, 3, 4, and 5, the IHP for pH 7.5R consistently demonstrated a slower rate of contraction. It was of interest to determine if this was related to the effect of the prior pH 6.5 solution or to the time for recording. Therefore, the preparation was recorded for three consecutive counts of 500, with two intermediate solution changes of pH 7.5. These results for four male adults are represented in Figure 10. During the second recording period (500-1000 cts) there was shift of the rate of frequency to slightly slower rate. During the third phase (1000-1500 cts), the peak rate of frequency remain constant with a greater number of responses at this rate. Thus the slower rate of frequency observed for pH 7.5R was related to the time at which the responses were measured.

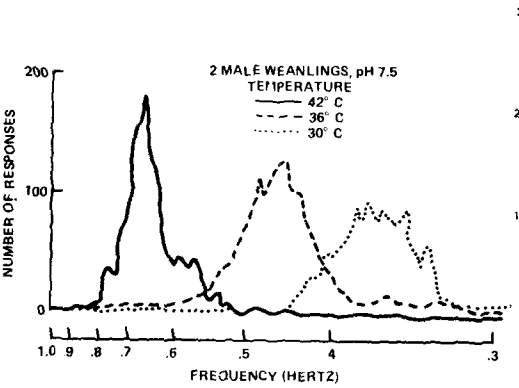

Fig. 8

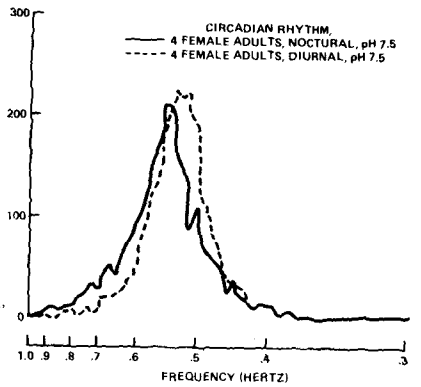

Fig. 9

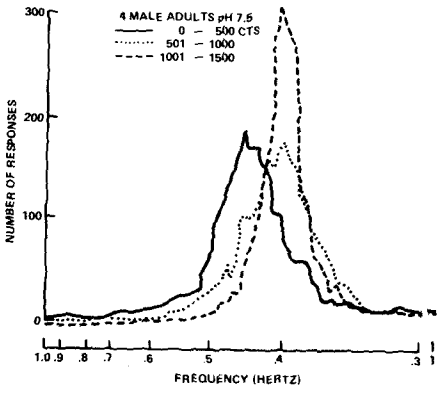

Fig. 10

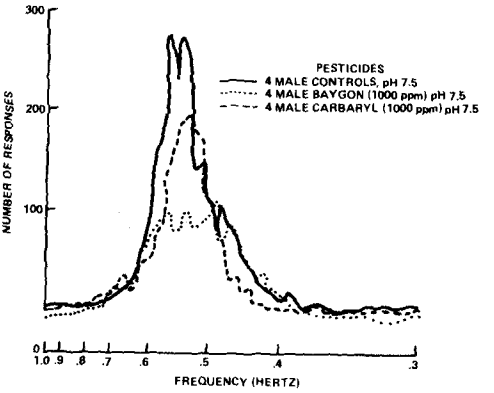

Fig. 11

Pesticides:

Forty-four day old white male rats were fed ab libitum either Baygon (1000 ppm) or Carbaryl (1000 ppm) in their feed for 3 weeks. The IHPs for these two groups are compared to controls in Figure 11. Even though no overt toxic symptoms were observed, the IHPs reflected an effect of the "in vivo" exposure. The Baygon appeared to be the more effective compound in producing random responses.

Conclusion

The "in vitro" recording of spontaneous contractions was selected as a neuromuscular screening method for "in vivo" exposure to pollutants. The rate of contraction was determined by interval histogram measurements. The method was assessed for effects of age, sex, pH, Ca^{++} concentration, temperature, and circadian rhythm. Male rats were shown to develop a slower rate of contraction with age. The rate of contraction was temperature and pH dependent. An effect was demonstrated for "in vivo" exposure to Baygon and Carbaryl at a level which did not produce overt symptoms.

ACKNOWLEDGMENT

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REFERENCES

BOZLER, EMIL. Physiological Reviews 42, 179 (1962).

SANTOLUCITO, J.A. and WHITCOMB, E.R. Brit. J. Pharmacol. 42, 298 (1971).