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Automative Quantification of Rat Duodenal Rhythmic Contraction

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In (1904) Magnus described the technique of using excised segments of intestines bathed by physiological salt solutions. Initially, the smooth muscle activity was recorded by mechanical methods. Today, in addition to the mechanical recording under isotonic, isometric, and auxotonic conditions, electrical activity is studied by intracellular and extracellular electrodes. Even though each method has limitations, the information obtained has resulted in advancement of smooth muscle physiology. However, the existing mechanical recording procedures have the following limitations (1) each smooth muscle preparation must serve as it own control, thus precluding "in vivo" exposures, (2) each evaluation of an elicited response is based primarily on visual impressions of the trace recordings, rather than automative quantitative analysis and (3) the primary parameter has been the contraction, either its presence, absence or magnitude. Studies on smooth muscle electrical activity and its relationship to contractions have suggested (1) that smooth muscle's electrical activity is composed of slow-waves and superimposed action potentials whose combined action is required to initiate a contraction, (2) that the slow waves(sometimes referred to as either basic electric rhythms or pacesetter potentials) originate in the longitudinal muscle's pacemaker cells and regulate the frequency of contractions, (3) that the superimposed action potentials determine the magnitude of contraction, (4) that both longitudinal and circular muscle layers are required for propagation of the electrical activity. In addition, Connor, et al (1976) have suggested that the slow waves are associated with cell metabolism by means of electrogenic ion transport. Thus, it appeared that "in vitro" measurements of the intact gut segment's frequency of contraction should be a suitable indirect method for monitoring smooth muscle activity, in particular, the slow-wave activity of its pacemaker cells. This approach was facilitated by using a microprocessor based system designed and constructed in this laboratory. Since the frequency of contraction is the reciprocal of the intercontraction interval, the latter was measured by the microprocessor based system. The automative quantitative measurements of 512 consecutive spontaneous contractions provided an intercontraction interval histogram for each gut segment similar to the interspike interval for neuronal spike trains (MacGregory & Lewis, 1971).

Representing each histogram by its first four cumulants resulted in expressing the gut's activity in terms of a mean, standard deviation, skewness and kurtosis. This in turn allowed a sample of gut segments to be defined. By subjecting the gut preparation to an environmental condition such as temperature which is known to affect metabolism and frequency, it was possible to evaluate how frequency in terms of the intercontraction interval reflected the change. An analysis of variance of regression on temperature indicated which characteristics of the intercontraction interval best reflected the smooth muscle's response to temperature. Thus, this study has proposed an approach which attempts to expand the usefulness of isolated gut segment in physiological and pharmacological studies by providing quantitative data for statistical analysis.

METHODS AND MATERIALS

CD male rats, 95-103 days of age, (Charles River Laboratories, Kinston, Mass.) were killed by cervical dislocation. A two-centimeter segment of the duodenum adjacent to the pylorus was removed and placed in cold modified-Ringer's solution, pH 7.5 (Whitcomb, 1978). The segment was flushed and freed from attached mesentery before tying each end. It was then allowed to equilibrate for one hour in 50 ml of modified-Ringer's solution at 7°C. The segment was transferred to 200 ml of modified-Ringer's solution and mounted for isometric measurements using a Statham force displacement transducer G7B-0.75-350, (Statham Instruments, Inc., Los Angeles, CA). The bathing solution was aerated continuously with compressed air and maintained at $\pm$0.5°C at either 33°, 36°, 39° or 42°C. Before recording, the segment was allowed to equilibrate thermally for one hour at the respective temperature. The transducer's output signal was sent to a Beckman Type RB Dynograph, (Beckman Instruments, Inc., Schiller Park, Ill). The amplified signal was then sent to the microprocessor-based system. This system determined the intercontraction interval for each of 512 consecutive rhythmic contractions. Thus an interval histogram representing the frequency distribution was obtained for each gut segment. The data was reduced by describing each histogram in terms of its first four sample cumulants, often called k-statistics (Kendall & Stuart, 1969). The respective roots (with sign retained) of the 2nd, 3rd and 4th k-statistics were used in the analyses, i.e. $k2P = (k2)^{1/2}$, $k3P = (k3)^{1/3}$ and $k4P = (k4)^{1/4}$.

The intercontraction interval of the rhythmic contractions was determined by software using a microprocessor based system designed and constructed in this laboratory. A block diagram of the system is shown in Figure 1. This system is based on the Intel Multibus (Intel Corp., 3065 Bowers Ave., Santa Clara, CA Santa Clara, CA. 95051), a SCI single board computer model 8010 (SCI Inc., 223 Crescent St., Waltham, MA 02154), a monolithic System 4502 memory board, and a special purpose interface board containing the circuitry necessary to provide the proper signals to the peripheral equipment. As indicated in the block diagram, the

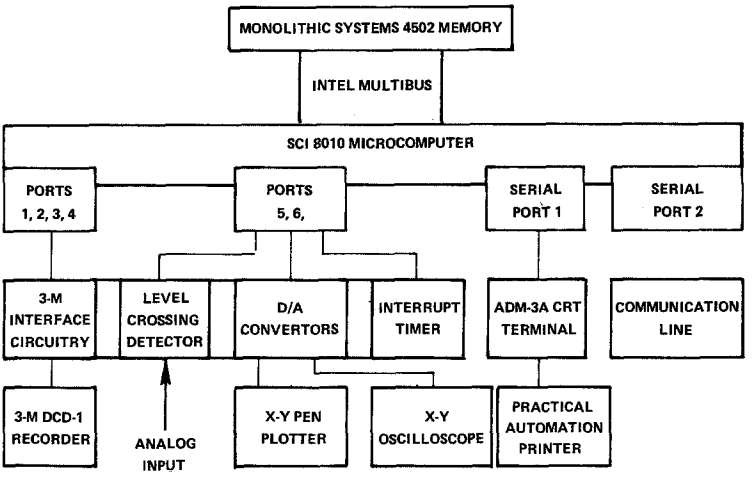

Figure 1 Time Interval Histogram Hardware System

computer board has six parallel ports and two serial ports. The parallel ports are used to interface the 3-M data recorder model DCD-1,(3M Company, 3M Center, St. Paul, Minn. 55101), D/A converters, and the level crossing detector. One serial port is used for optional communication to a remote computer. The special purpose interface board contains the interface circuits for the 3-M recorder, the D/A converters, the level crossing detector, and the interrupt timer circuit. Local printout is obtained on a serial printer connected to the CRT terminal.

The interrupt timer circuit generates pulses at 40 Hz. These pulses are fed to the interrupt input on the computer and causes interrupts to occur in the computer program execution. The D/A converters are the interface between the microcomputer and the analog plotting instruments consisting of an X-Y plotter and an oscilloscope. The data to the X-Y plotter can be turned on and off under software control in the software plotting routines. The level-crossing detector is a comparator circuit which determines the zero (or other adjustable level) crossings of an input signal. A software routine under interrupt control counts the number of 40 Hz pulses that occur between consecutive crossings of the input signal.

The 3-M interface is a collection of special interface circuits which provide the particular signal characteristics required by the DCD-1 cartridge recorder. The interface consists of inverters, one hot multivibrators, and logic gates to satisfy the requirements of the DCD-1 timing and status lines.

RESULTS AND DISCUSSION

Each gut-segment's rhythmic contraction pattern was characterized by a mean (k1P), standard deviation (k2P), skewness (k3P) and kurtosis (k4P) based on 512 consecutive intercontraction interval measurements. Each temperature group's response, summarized by an average (k) and standard deviation of k1P, k2P, k3P and k4P, is listed in Table 1.

Table 1. Mean and Standard Deviation of the Intercontraction Interval k-Statistics by Temperature Group

	Temperature °C			
$\bar{k}$-statistics	33(6)	36(22)	39(8)	42(13)
$\bar{k}$1P	2496 ± 145	2181 ± 258	1729 ± 201	1481 ± 145
$\bar{k}$2P	511 ± 145	537 ± 243	477 ± 232	321 ± 326
$\bar{k}$3P	491 ± 317	518 ± 263	655 ± 177	358 ± 399
$\bar{k}$4P	814 ± 128	655 ± 443	611 ± 510	414 ± 387

( ) = number of observations
k1P = mean (msecs), k2P = standard deviation, k3P = skewness, k4P = kurtosis

While age must be considered a factor in physiological responses, in this study the age difference between 95-103 days was assumed to be negligible.

The mean ($\bar{k}$1P) and standard deviation for each temperature group is listed in Table 1. Analysis of variance indicated that the $\bar{k}$1P of the intercontraction interval reflected a significant ($p<.0001$) linear affect due to temperature. A subsequent regression analysis is shown in Figure 2, and Table 2.

The mean ($\bar{k}$2P) and standard deviation for each temperature group is listed in Table 1. An analysis of variance suggested that $\bar{k}$2P did not reflect an affect ($p=0.1286$) due to temperature.

The mean ($\bar{k}$3P) and standard deviation for each temperature group is listed in Table 1. An analysis of variance suggested that $\bar{k}$3P did not reflect a temperature affect ($p=0.1132$).

The mean ($\bar{k}$4P) and standard deviation for each temperature group is listed in Table 1. An analysis of variance suggested that $\bar{k}$4P did not reflect an affect ($p=0.2227$) due to temperature.

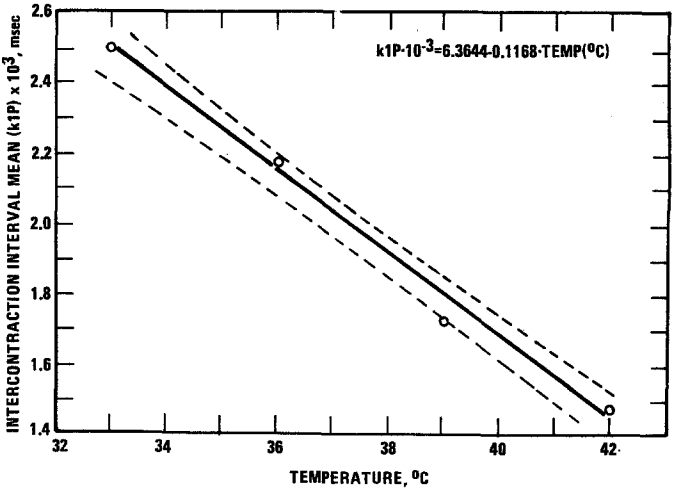

Figure 2 Linear Regression of Intercontractions Interval Mean (k1P) on Temperature with 95% Confidence Limits k1P x 10^3 = 6.3644 - 0.1168 x T°C

Table 3. Analysis of Variance of Regression of Intercontaction Interval Mean (x 1000) on Temperature

SOURCE	DOF	SS	MS	F	P
Regression	1	6.142	6.142	136.998	.0000
Error	47	2.107	.045		
Lackfit	2	.069	.035	.764	.4715

SUPPLEMENTARY STATISTICS

$\overline{X}$ = 37.71 Y = 1.900 SE(B1) = .010
B1 = - .117 BØ = 6.366 SE(BØ) = .378
R-Square = .745 C.V. = .108

DOF = Degrees of Freedom SS = Sum of Squares MS = Mean Square
X = Temperature B1 = Estimated Slope BØ = Estimated Intercept
Y = Intercontraction Interval C.V. = Coefficient of Variation

The exploratory nature of this study required empirical choices. The first choice was the selection of 512 intercontraction intervals as a sampling size for each gut segment. The second choice was the selection of 25 msec. increments to measure the intercon-

traction intervals. Preliminary studies had indicated that at 36°C the interval was approximately 2000 msecs. Since the 25 msec. increment was 1.25% of this interval, it was assumed to be adequate. The third choice was a method of reducing the 512 intercontraction interval measurements on each duodenal segment to a manageable yet meaningful parameter set. Various continuous distributions including the normal, lognormal, Weibell, and gamma were fit to a sample of the histograms. Although the gamma generally fit best in terms of the estimated log-likelihood function, none of these distributions fit very well when measured by the traditional X^2 goodness-of-fit test. Finally, it was decided to describe each duodenal segment's histogram by its first four sample cumulants or k-statistics. Although they are analagous to moments, k-statistics are invariant (except for the mean) to a shift in the origin of the distribution. Thus, the use of k-statistics will allow the analyses of skewness and kurtosis for different duodenal segments, even though the means for these segments are different. Only the first four moments were used since higher moments are overly sensitive to sampling function (Kendall & Stuart 1969). The fourth choice was the selection of an exogenous stimulus to evaluate how affective the intercontraction interval approach quantified the response of the pacemaker activity. Temperature seemed to be a reasonable choice because (1) it could be controlled accurately, (2) it had been demonstrated that increasing temperature will affect metabolism and frequency (3) within a limited range temperature would not damage the smooth muscle. Since no consistent temperature has been used in smooth muscle studies, 33°, 36° and 39°C were selected to bracket body temperature. A temperature of 42°C was selected as the highest temperature feasible with a one hour thermal equilibration. The fifth choice was the selection of a bathing volume. In this study a volume of 200 ml was used to diminish the possible affect of acetylcholine that is released constantly by the tissue. Finally, the sixth choice was the selection of a laboratory species. On the basis of the studies and reviews cited, the male albino rat would not have been the animal of choice. However, because of cost and ease of handling the male albino rat was selected.

While it is known that increasing temperature results in a faster rate of contraction, the form of this relationship was defined quantitatively for the rat's duodenal segment by this study. The analysis of the intercontraction interval mean strongly suggested that this relationship is linear over the range of 33° to 42°C. The estimated slope indicated that for each 1°C increase there was an intercontraction interval decrease of 117 msecs. This regression line explained 74.5% of the variation present in the k1Ps. That the mean ($\overline{k1P}$) was the only k-statistic which reflected a significant affect does not diminish the value of the other three k-statistics. It is possible that sample size in each temperature group resulted in insufficient power to detect differences in k2P, k3P and k4P. This study has shown that the intercontraction interval distribution departs from a symmetrical form

by being skewed to the right. The skewness (k3P) like the standard deviation (k2P) was not altered significantly by temperature.

Finally, the intercontraction interval histogram was characterized by leptokurtic distribution. That is, when compared to a normal distribution it had a narrower modal position and higher tails. Just as with standard deviation and skewness, kurtosis (k4P) did not reflect a significant affect of temperature as a group.

Now that it has been demonstrated that a parameter of smooth muscle activity can be quantified, what is the potential value of this this procedure? That a given gut segment and sample of gut segments can be characterized eliminates the restriction that each gut segment must serve as its own control. Now in vivo smooth muscle exposure can be evaluated by an in vitro mechanical recording method. How well does the proposed parameter reflect the response of the system? In the present study 74.5% of the variability attributed to the effect of temperature was explained. Systems in addition to the electrical slow-waves contributed to the final time course as measured by the intercontraction interval. However, unless these systems responded to temperature comparable to the pacemaker cells metabolism, it would appear that the frequency as measured, primarily reflected the electric slow-wave activity. Diamant and Bortoff (1969) have reported "...that the slow-wave frequency recorded from segments of intestines in vitro is identical to the frequency of that segment in sites in the absence of any myogenic influence from above. In other words, this frequency may be close to, if not, the same as the intrinsic frequency of that segment of intestine." By monitoring the frequency of smooth muscle spontaneous contraction, as suggested in this study, the intrinsic response of the smooth muscle tissue can be evaluated by the analysis of automative quantitative data for a given condition. This expands the usefulness of the smooth muscle preparation as a physiological and pharmacological tool.

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