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INFLUENCE OF SEX AND SEXUAL HORMONES IN THE BRADYKININ–RECEPTOR INTERACTION IN THE GUINEA PIG ILEUM*

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Abstract—Strips of the ileal longitudinal muscle layer of guinea pigs have the following affinities for bradykinin: in males, $K_n = 1{\cdot}55 \times 10^{-8}$ M with S. D. $= 0{\cdot}23 \times 10^{-8}$ M; in females, $K_n = 0{\cdot}39 \times 10^{-8}$ M with S. D. $= 0{\cdot}04 \times 10^{-8}$ M. No difference between males and females was found in the K_n values for acetylcholine or for histamine. Castration of males and hormonal treatment after castration modify K_n values for bradykinin: in castrated males, $K_n = 0{\cdot}84 \times 10^{-8}$ M with S. D. $= 0{\cdot}01 \times 10^{-8}$ M; in castrated males treated with testosterone, $K_n = 1{\cdot}33 \times 10^{-8}$ M with S. D. $= 0{\cdot}34 \times 10^{-8}$ M; and in castrated males treated with β-estradiol, $K_n = 0{\cdot}45 \times 10^{-8}$ M with S. D. $= 0{\cdot}02 \times 10^{-8}$ M. No difference was observed in the maximum contractility to bradykinin among the five groups of animals. The state of the bradykinin receptors in the castrated animals seems to be influenced by the sexual hormones in the following way: administration of testosterone to the animal changes the receptors to a state of lower affinity for bradykinin; administration of β-estradiol changes the receptors to a state of higher affinity for bradykinin. The results of the hormonal effects were discussed in terms of an allosterically controlled receptor or the synthesis of modified receptors.

The drug–receptor dissociation constant (K_n) is an important parameter used to describe the interaction between drugs and receptors. Our studies to determine K_n values for bradykinin in the longitudinal muscle strip (LMS) of guinea pig ileum show an original behavior of the structure that rendered possible the observation of sexual difference in the affinity to bradykinin, whereas no such difference exists in the affinities to acetylcholine and histamine [1].

Hormonal influences in responses of smooth muscle were described in masculine genital apparatus [2–5] and in the uterine muscle [6–8], but no report of such influence had been made in the case of guinea pig ileum. It is clear to us that such differences could be important in quantitative work in the field, and the aim of this paper is the description of hormonal influence in the affinity of LMS to bradykinin.

MATERIALS AND METHODS

Guinea pigs weighing from 500 to 700 g were used. A portion at about 10 cm of the terminal ileum was removed immediately after stunning and exsanguination the animals. The piece of ileum was washed with nutritive solution and prepared according to the method of Rang [9] for separation of the longitudinal muscle strip. A piece of LMS about 3·8 to 4 cm long (approximately 25 mg tissue) was suspended in an organ bath at 36°.

In order to retard the diffusion of the agonists out of the organ bath, it was constructed with independent and constricted ports for the admittance and withdrawal of the perfusion fluid. The volume of the perfusion fluid in the organ bath was rigorously kept at 10 ml through the use of a syringe intercalated between the reservoir and the heating coils.

The perfusion fluid was Tyrode's solution from which sodium bicarbonate was omitted and with monosodium phosphate used in a concentration of 0·50 g/liter. The pH of the solutions was raised with 0·4 M NaOH to 7·4 during the preparation. After preparation of the Tyrode's solution, the osmolarities were measured in a Fiske osmometer, model G-66; the values obtained were from 270 to 280 mOsmoles/liter. The bath fluid was continuously bubbled through with air in the organ bath.

The hormones used were testosterone propionate and β-estradiol dipropionate, both from Sigma Chemical Co. As agonists we used acetylcholine chloride and histamine phosphate, also from Sigma Chemical Co., and bradykinin from Schwartz/Mann. Solutions of the agonists were prepared in Tyrode's solution immediately before use, by dilution of stock solutions maintained at −30° in little flasks that contained just the volume necessary for one experiment. We made as many solutions as necessary so as to add to the organ bath the same volume (0·1 ml), even when different concentrations of agonist were required. Each dose of agonist was added to the organ bath 1 min after washing out the previous dose. The agonists were used in increasing concentrations, and we made triplicate or quadruplicate recordings for each dose. We chose the doses so as to obtain an arithmetic progression in the values of the reciprocal of the doses [10].

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Contractions were recorded on a smoked drum with an auxotonic lever, prepared according to Paton [11], that gave a 6 × magnification, had a resting tension of 300 mg, and an action load of 150 mg/cm of deflection on the drum.

After each experiment the recordings were measured with a caliper rule and the values of the responses were related to the respective values of agonist molar concentration through Clark's equation [12] in double reciprocal form [13]. The equation used was

$$\frac{1}{Y} = \frac{1}{Ym} + \frac{K_n}{Ym}\,\frac{1}{X} \qquad (1)$$

in which Y = the value of one response to dose X, X = the molar concentration of agonist, Ym = the maximum contraction height and K_n = the dissociation constant of the agonist–receptor complex (reciprocal of Clark's constant). The values of Ym and K_n were calculated with the help of an IBM 1130 computer, using a program KMVM [14]. To minimize the errors in Y obtained at low agonist concentrations, we introduced in the calculations the variance as a statistical weight [15]. Comparison between mean values of K_n was made using Student's t-test. Comparison between the values of maximum contraction heights calculated in every experiment was performed by analysis of variance.

In an attempt to detect a direct hormonal influence on the affinity of the LMS for bradykinin, we first measured a complete series of contractions with acetylcholine and another series with bradykinin; then, we substituted for the normal Tyrode's solution another one containing hormone in a concentration of $1{\cdot}0 \times 10^{-7}$ M. Stock solutions of the hormones were prepared in ethanol, at a concentration of 1 mM, and aliquots of these stock solutions were added to the Tyrode's solution in order to obtain the desired final concentrations. After adding hormone to the Tyrode's solution we changed the perfusion fluid in the organ bath at 5-min intervals over a period of 1 hr; then, we repeated all the determinations using the perfusion fluid with hormone.

Castrations were performed under aseptic conditions in male guinea pigs anesthetized with pentobarbital (3 mg/100 g of weight), using scrotal access for the surgery. After 30 days the animals were divided randomly into three groups. Each animal of group A was injected subcutaneously with 0·2 ml of an oil solution of testosterone propionate (2·5 mg/ml) every day for 15 days. The animals of group B were treated with β-estradiol dipropionate for 30 days, receiving a subcutaneous injection of 0·2 ml of an oil solution of the hormone (0·1 mg/ml) on alternate days. The animals in Group C did not receive hormone. After the treatments the animals were sacrificed in the manner described and the values of K_n were determined in LMS.

RESULTS

Dissociation constants of drug receptor complexes for bradykinin, acetylcholine and histamine. The experimental data obtained were of the pattern shown in Fig. 1. The mean values of K_n for bradykinin found for male and female guinea pigs are given in Table 1. Between these means there exists a statistically significant difference at the level of $P < 0{\cdot}001$. Figure 2 shows the regression lines calculated for determinations of K_n for bradykinin in male and female guinea pigs. K_n values for acetylcholine and histamine are also shown in Table 1. For both agonists, the differences between the means found for males and females are not statistically significant at the level of $P < 0{\cdot}05$.

In six animals, three of each sex, we determined K_n values for acetylcholine and bradykinin in the same piece of LMS. A comparison between these values is shown in Table 2. For male guinea pigs, the quotient K_n (acetylcholine)/K_n(bradykinin) is about 1; for female guinea pigs, this quotient is about 6.

Dissociation constants obtained using hormones in the perfusion fluid. The K_n values for bradykinin and for acetylcholine found with testosterone-perfused LMS from female guinea pigs were identical to the controls without testosterone. Again, no change in K_n values for these agonists was observed with β-estradiol-perfused LMS from male guinea pigs.

Effect of castration on the values of the dissociation constants of the bradykinin–receptor complex. The castrated guinea pigs of group C were sacrificed 30 days after castration. The values found for the dissociation constants of the LMS bradykinin receptor complex are given in Table 3. The mean value is significantly different from the mean found for females ($P < 0{\cdot}001$)

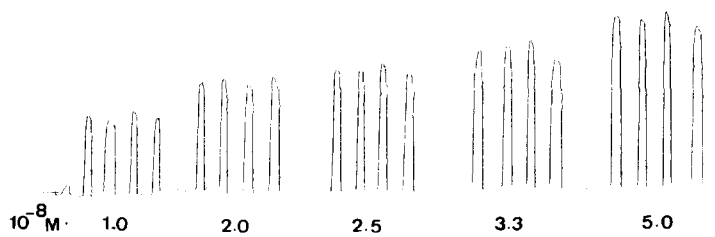

Fig. 1. Experimental data obtained in LMS for the calculation of one K_n value from a normal male. Experimental conditions are as described in Methods.

Table 1. Mean values of drug–receptor dissociation constants calculated for brady-kinin, acetylcholine and histamine in female and male LMS*

Agonist	Females	Males
Bradykinin	$3{\cdot}87 \times 10^{-9} \pm 0{\cdot}44 \times 10^{-9}$ M (9; 180)	$15{\cdot}47 \times 10^{-9} \pm 2{\cdot}26 \times 10^{-9}$ M (6; 99)
Acetylcholine	$1{\cdot}82 \times 10^{-8} \pm 0{\cdot}24 \times 10^{-8}$ M (8; 143)	$1{\cdot}56 \times 10^{-8} \pm 0{\cdot}23 \times 10^{-8}$ M (7; 121)
Histamine	$1{\cdot}24 \times 10^{-7} \pm 0{\cdot}26 \times 10^{-7}$ M (4; 83)	$1{\cdot}31 \times 10^{-7} \pm 0{\cdot}25 \times 10^{-7}$ M (9; 153)

* Following each mean are the standard deviations of the means. In parentheses are: first, the number of K_n determinations that originated the mean values; and second, the total number of contractions measured.

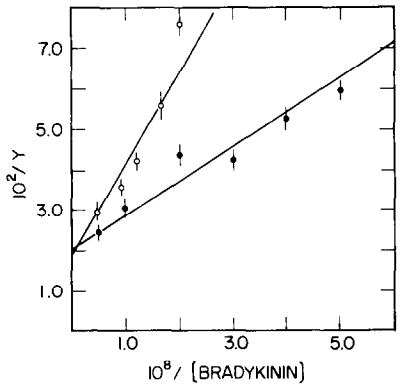

Fig. 2. Regression lines obtained in calculating K_n for bradykinin in female (●— ●) and in male (○— ○) LMS. The points are means of reciprocals of contraction heights at each agonist concentration; the vertical lines are ranges of such reciprocals. In the actual calculation of K_n, all points were used.

and from the mean found for noncastrated males ($P < 0{\cdot}05$).

Effect of testosterone treatment on the values of the dissociation constants of the bradykinin–receptor complex. The castrated guinea pigs that were given testosterone were sacrificed at the end of the treatment. The values found for the dissociation constants of the bradykinin–receptor complex are given in Table 4. The mean value is not significantly different from the mean value found for noncastrated males, but differs significantly from the mean value found for females ($P < 0{\cdot}005$). The mean value is not significantly different from the mean value found for untreated, castrated males at $P < 0{\cdot}05$, but differs significantly at the level of $P < 0{\cdot}10$.

Table 3. K_n values calculated for bradykinin in LMS of castrated males 30 days after castration

Expt. No.	No. of points*	$10^9 \cdot K_n$ (M)
38	14	7·03
39	13	11·14
40	12	6·61
41	12	6·88
42	12	10·43
Mean		8·42
S. D. of mean		0·98

* Number of contractions measured to obtain each K_n value.

Effect of estradiol treatment on the values of the dissociation constants of the bradykinin–receptor complex. After β-estradiol treatment of the castrated guinea pigs of group B, they were sacrificed and the values of K_n for bradykinin were determined in the LMS. The values found are given in Table 5. There is no difference between the mean value found for this group and the mean value found for females, but a statistically significant difference does exist between group B and group C ($P < 0{\cdot}05$) and between group B and untreated males ($P < 0{\cdot}005$). A comparison of the results is shown in Fig. 3 as a histogram.

Calculated values of maximum contractions of LMS to bradykinin. The values of maximum contractions calculated for bradykinin were grouped (Table 6) and compared by means of an analysis of variance that showed no statistical difference between the five groups at the level of $P < 0{\cdot}05$.

Table 2. Comparison between the values of K_n for bradykinin and acetylcholine in the same piece of longitudinal muscle

Sex	Expt. No.	Acetylcholine $10^8 \cdot K_n$ (M)	Bradykinin $10^8 \cdot K_n$ (M)
	13	1·25	1·59
Males	14	1·29	0·97
	15	1·63	1·66
	7	2·49	0·36
Females	8	1·30	0·33
	9	1·90	0·25

Table 4. K_n values calculated for bradykinin in LMS of castrated males after 15 days of testosterone treatment

Expt. No.	No. of points	$10^9 \cdot K_n$ (M)
43	12	24·41
44	12	9·59
45	12	17·47
46	12	4·93
47	15	10·03
Mean		13·29
S. D. of mean		3·43

Table 5. K_n values calculated for bradykinin in LMS after 30 days of β-estradiol treatment

Expt. No.	No. of points	$10^9 \cdot K_n$ (M)
48	14	5·09
49	16	4·59
50	18	4·13
51	9	4·24
Mean		4·51
S. D. of mean		0·22

DISCUSSION

The thermodynamics of the bradykinin receptor interaction in the LMS were found to be dependent on the sex and on the hormonal conditions of the guinea pigs. The female preparation has about four times more affinity for bradykinin than the male preparation. Such sexual difference does not exist for acetylcholine or for histamine.

Sexual differences in affinity for groups of agonists have been described in structures associated with the genital apparatus by several authors [2, 5, 16]. Our results show sexual differences and a hormonal influence in the affinity of the guinea pig ileum only for bradykinin, and not for histamine or acetylcholine. This leads to the hypothesis that a physiological role may be associated with the sexual differences in the affinity of the smooth muscle of the guinea pig ileum for bradykinin.

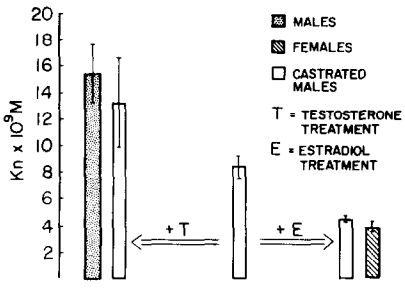

Fig. 3. Comparison of the magnitudes of K_n for bradykinin in the five experimental groups described in the text.

Our results fo not reveal differences in the contractile capacity of the longitudinal muscle stimulated by bradykinin. In preparing the pieces of longitudinal muscle strip we tried to use pieces of similar length, from 3·8 to 4 cm. After the experiments the humid preparations were weighed and their weights were always close to 25 mg. Thus, we considered that it was valid to compare the calculated maximum values of contractility. The statistical analysis shows that sex or hormonal state of the animals does not modify the maximum values. This observation suggests that the LMS does not behave like the rat seminal vesicle [5] or like the rabbit uterus [7], in which the contractile capacity is modified by the hormonal condition of the animal. Moreover, it can be inferred from our results that castration and hormonal treatment do not modify the actomyosin content of the LMS, as it does in the rabbit uterus [17].

Since no influence on contractility was seen, our findings seem to be directly related to the drug receptor interaction or to the receptor structure. Two alternatives should be considered in this respect: the first is that modified receptors are being synthesized as a consequence of hormonal effects; and the second is that different conformational states of the bradykinin receptor could be stabilized by sexual hormones, so as to bring about the differences in affinity observed. Our data do not permit a decision concerning these alternatives. However, additional data point to the fact that the interaction phenomenon between drug and receptor remains essentially unaltered, despite the finding that different affinities were found under the experimental conditions tested. Thus, determination of the molecularity of the interaction, using the equation of Johnson *et al.* [18], showed that with the various preparations used the interaction was always unimolecular, as given by a slope close to 1·0 in the equation:

$$\log|(Ym - Y)/Y| = \log K_n - n \log X \qquad (2)$$

A speculative model of the second alternative requires that at least four conformations should exist for the bradykinin receptor in LMS, three of which would respond actively to bradykinin. Each of the three would have a different affinity for bradykinin and their interconversion equilibria would be controlled by the sexual hormones. Thus, the K_n values measured in LMS obtained from animals under

Table 6. Comparison between the maximum contraction heights (Ym) calculated for bradykinin in LMS in the five experimental groups*

Untreated males	Untreated females	Castrated males not treated with hormones	Testosterone-treated castrated males	β-Estradiol-treated castrated males
18·71	51·70	56·57	42·33	38·88
50·22	46·01	44·93	49·74	40·75
20·90	51·48	46·44	46·44	60·65
40·15	48·25	45·86	37·85	35·06
40·32	54·81	34·82	29·48	
44·95	53·91			
	41·80			
	41·96			
	35·09			
35·87	47·22	45·72	41·17	43·84

* Values of Ym are given in mm. The means of the groups are at the bottom of the table.

definite experimental conditions, such as normal male or female, or castrated treated, or castrated untreated, would reflect the prevalence of a given conformation, compatible with a definite hormonal balance. An allosteric behavior has indeed been proposed by Changeux *et al.* [19] and Karlin [20] for receptors found in cell membranes, which would exist in two conformations, one active and the other inactive.

Our experiments in which hormones were added to the perfusion fluid failed to show any differences in affinity of the receptor for bradykinin. The ineffectiveness of action *in vitro* might be due to a requirement for hydrolysis of the esters for activity. We do not know, in addition, if the appropriate levels of hormone concentration were attained at the receptor sites, nor if the time of incubation required to affect the receptor was sufficient.

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