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Loss and recovery of sensitivity of guinea-pig isolated ileum to the spasmogenic action of the complement peptide $C5a_{desArg}$

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1 Deactivation (tachyphylaxis) of the guinea-pig isolated ileum to the spasmogenic action of the complement peptide $C5a_{desArg}$ was analysed.

2 It appeared to consist of 2 components: a fast one, characterized by rapid onset of deactivation and by recovery within 2–3 min (see Damerau *et al.*, 1985b), and a slow component, characterized by progressively increasing loss of sensitivity (until complete deactivation after several minutes) and by recovery within about 80 min.

3 Slow deactivation shows an exponential time course; it is dependent on concentration as well as contact time with $C5a_{desArg}$ and occurs under conditions (incubation in Ca^{2+}-free medium or at 16°C) in which the peptide has no spasmogenic effect.

4 Recovery from slow deactivation follows an exponential time course at 34°C but is blocked at 16°C; on average it reaches about half of the initial sensitivity.

5 The results indicate that the slow deactivation is mainly due to blockade of C5a receptors by the ligand and is independent of the spasmogenic effect of $C5a_{desArg}$.

Introduction

The complement peptides C3a and C5a induce contractions of a number of smooth muscle organs. This effect has been demonstrated in vascular, gastrointestinal and airway preparations from different animal species, for example tracheal rings, strips of isolated ileum or uterus from guinea-pigs as well as pieces of rat colon, ileum or stomach (for reviews see Vogt, 1974; Hugli & Müller-Eberhard, 1978). Hog $C5a_{desArg}$ which was earlier described as being inactive (Hugli & Müller-Eberhard, 1978) is now known to be spasmogenic (Damerau *et al.*, 1980; Gerard & Hugli, 1981).

The spasmogenic action of the C5-peptides on guinea-pig ileum is partially mediated by endogenous histamine which is released from serosal mast cells upon stimulation, although histamine-independent mechanisms also contribute (Bodammer & Vogt, 1970; Sorgenfrei *et al.*, 1982). Their nature is still unknown, and the question is open as to whether they represent a direct action of the C5-peptide on the smooth muscle cells. The proposed role of histamine in guinea-pig ileum was derived from the following observations: $C5a/C5a_{desArg}$ led to degranulation of mast cells and release of histamine (Mota, 1959; Bodammer & Vogt, 1970; Johnson *et al.*, 1975), the latter providing histamine concentrations sufficient to induce contraction (Sorgenfrei *et al.*, 1982). Furthermore, their spasmogenic effect is markedly inhibited by H_1-antihistamines (Hahn & Oberdorf, 1950; Bodammer & Vogt, 1970; Sorgenfrei *et al.*, 1982).

All tissues contracted by $C5a/C5a_{desArg}$ have in common the fact that they gradually lose their sensitivity to the spasmogenic action of the complement peptides in the course of repeated applications or with advancing incubation time, i.e. they undergo tachyphylaxis or – as it will be termed here – deactivation (Friedberg *et al.*, 1964; Vogt *et al.*, 1969; Bodammer & Vogt, 1970). Deactivation by C5-peptides has also been observed in leukocytes and platelets (Grossklaus *et al.*, 1976; O'Flaherty *et al.*, 1979). In leukocytes, it has been assumed to be due to blocking of specific receptors, as C5a and $C5a_{desArg}$ bind to them with high affinity and cannot be removed to any measurable degree by washing (Chenoweth & Hugli, 1978). Receptor binding and consequent changes of the leukocytes' functional state (e.g. chemotactic response, exocytosis or re-

lease of oxygen radicals) cannot be continuously measured and correlated. We have therefore investigated deactivation by hog $C5a_{desArg}$ in segments of guinea-pig isolated ileum, as it allows repeated stimulation and continuous recording of the responses in the same tissue. Furthermore, incubation conditions can also be easily changed several times for the same ileum segment. However, it should be stressed that it is not yet clear which cell types are stimulated when $C5a_{desArg}$ acts on the ileum, although mast cells are clearly involved.

In this paper we will describe the basic kinetic parameters of (mainly slow) deactivation, and of the recovery therefrom, in the guinea-pig isolated ileum, such as dependence on time, temperature, concentration, frequency of stimulation and Ca^{2+} ions. In the two following papers the two components of deactivation will be analysed in detail by pharmacological means (Damerau *et al.*, 1985a,b).

Methods

$C5a_{desArg}$

Generation and purification of the complement peptide $C5a_{desArg}$ obtained from yeast-activated hog serum has been described in detail by Zimmermann *et al.* (1980). The final peptide material was pure in acid disc electrophoresis and formed only one band against rabbit anti-$C5a_{desArg}$ antiserum, but no band against anti-hog serum or anti-C3a antisera.

Determination of isometric contractions

Segments of isolated ileum (about 2.5 cm in length) from guinea-pigs weighing 300–400 g were mounted in an organ bath of 6.3 ml capacity and connected to a strain gauge isometric recording system (basal tension 1 g). The medium was Tyrode solution at 34°C, aerated with 95% O_2 and 5% CO_2. After 30 min incubation, acetylcholine (ACh) was applied several times to obtain constant reactivity. When not otherwise stated, the normal test rhythm, in which $C5a_{desArg}$ alternating with two ACh applications was given, obeyed the following time schedule: change of bath fluid at zero time; injections of spasmogenic substance after 60 s; change of bath fluid after the intervals indicated in the legends to the figures (ACh was usually removed after 15 s) = zero time of the next cycle. The total cycle time, between one application of $C5a_{desArg}$ and the next, was usually 240–270 s; modifications are described in the legends to the figures.

The sequence of the different experimental treatments was alternated from experiment to experiment. The motor response was expressed in terms of the effect of the first response to $C5a_{desArg}$ or of the maximal effect of ACh which was taken as 1.0 (maximally active ACh concentrations ranged from $1-3 \times 10^{-5}$ M).

Determination of mast cell degranulation

Ileum segments were stimulated once with 0.08 and once with 0.16 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (contact times 120 s) or as a control with ACh only, either at 34°C or at 16°C. After fixation in a mixture of 72% ethanol and 10% formaldehyde containing 1% calcium acetate for 2 h and mast cell staining with an acidified ethanolic solution of toluidine blue for 24 h (Smith & Atkinson, 1956), the tissues were washed with 95% ethanol for about 60 min.

Strips of the superficial ileum layers were carefully cut off and mounted between microscopic slide and cover glass. Intact and degranulated subserosal mast cells were counted by transmission microscopy at 500 × magnification in 25 fields per test tissue (containing 50–60 mast cells in total).

Curve fitting

The data shown in Figure 1 were linearly fitted with least squares of deviation according to the formula $y(x) = a \cdot e^{bx}$; the values of the spasmogenic effects measured (means as well as results of the individual experiments) are represented by 'y', the number of the respective $C5a_{desArg}$ applications by 'x'. The data from each individual experiment shown in Figure 5 were fitted non-linearly with least squares of devia-

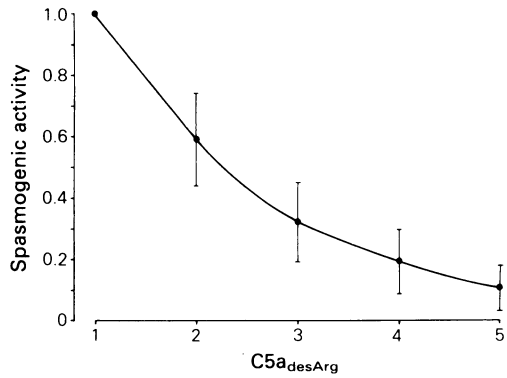

Figure 1 Deactivation of guinea-pig isolated ileum segments by repeated applications of 0.05 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (contact time of 30 s each; number of applications, abscissa scale). $n = 100$; mean values with s.d. shown by vertical lines. Ordinate scale: spasmogenic activity related to the first submaximal response to $C5a_{desArg}$ in the individual experiment.

tion according to the function $f(t) = a \cdot (1 - e^{bt})$ in which 'a' shows the maximum recovery reached in these experiments and 'b' is the inverse relaxation time. These calculations were kindly performed by Dr I. Pardowitz from our institute.

Results

(1) *Kinetics of deactivation induced by repeated applications of $C5a_{desArg}$*

Figure 1 shows the gradual decrease in the strength of the contractions of guinea-pig isolated ileum segments, induced by repeated applications of $C5a_{desArg}$ ($0.05\ \mu g\ ml^{-1}$). The deactivation curve includes the results of 100 experiments which were compiled in the following way: the first stimulation with $C5a_{desArg}$ causing a submaximal contraction (less than 90% of the effect of a supramaximal ACh concentration) was taken as the first application and allocated a relative spasmogenic activity of 1.0 in each individual experiment. Furthermore, only results of experiments were used in which the 5th peptide application (determined in this way) still induced a measureable response, namely >0.05 relative spasmogenic effect. The purpose of this kind of compilation was to avoid deformation of the deactivation curve in its initial and final part (such as that seen in Figure 6).

The motor responses of the test tissues were almost halved by each application, so that by the 5th test it had decreased to about 10% of the initial sensitivity (Figure 1). Curve fitting of mean values (Figure 1) led to the equation $y = 1.75\,e^{-0.56x}$ (correlation coefficient $r = 0.9997$) or, when using the 500 individual test values, to $y = 1.87\,e^{-0.6x}$ ($r = 0.85$). This suggests that loss of sensitivity to the spasmogenic action of $C5a_{desArg}$ proceeds in a strictly exponential form.

The course of deactivation was correlated with the period of time the test tissues had been in contact with $C5a_{desArg}$ (= cumulative contact time). Ileum segments treated repeatedly for 30 s were deactivated more slowly than others incubated 45 s each time, and much more slowly than segments exposed to $C5a_{desArg}$ for 90 s (Figure 2b). When the spasmogenic effects measured were plotted against the cumulative contact times, all points assembled near an exponential curve as shown in Figure 2a: the sensitivity of the ileum segments fell to 50% after a cumulative contact time of about 60 s, and further down to 10% after about 200 s.

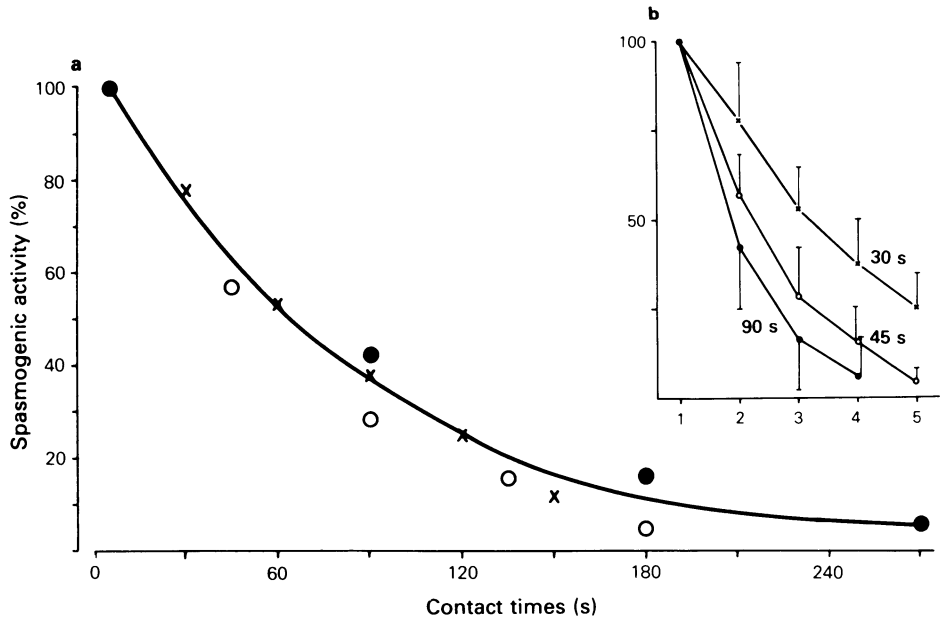

Figure 2 (a) Dependence on the cumulative contact times (in s, abscissa scale) of deactivation with $C5a_{desArg}$ applied repeatedly in a concentration of $0.05\ \mu g\ ml^{-1}$; individual contact times 30 (×), 45 (○) or 90 (●) s. Ordinates (of a and b): spasmogenic effect of $C5a_{desArg}$ given as % of the first application (= 100%) of each experiment. $n = 6$; mean values with s.d. shown by vertical lines. (b) Deactivation by repeated applications (abscissa scale) of $C5a_{desArg}$ with different contact times. Results from the same experiments as in (a).

(2) *Effect of concentration on rate of deactivation*

Ileum segments which were repeatedly stimulated by 0.02 $\mu g\,ml^{-1}$ $C5a_{desArg}$ for 30 s still responded weakly to the 10th peptide application (Figure 3, upper curve). At a ten fold higher concentration $C5a_{desArg}$ already induced complete deactivation after the 6th application, and at 2 $\mu g\,ml^{-1}$ $C5a_{desArg}$ the ileum segments lost their sensitivity after 3 applications. Hence, increasing concentrations of $C5a_{desArg}$ very much accelerated the course of deactivation.

(3) *Lack of dependence of deactivation on actual contractions of the ileum*

Segments of ileum which had been fully deactivated to 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$ given ten times still responded well to a subsequently applied concentration of 0.2 $\mu g\,ml^{-1}$. However, when the series of treatments with the lower dose was prolonged to 15 applications the subsequent test with 0.2 $\mu g\,ml^{-1}$ produced a much smaller contraction (40% of that after 10 pre-exposures). Hence, the deactivation process was independent of contractions which in both series had subsided after the 9th application.

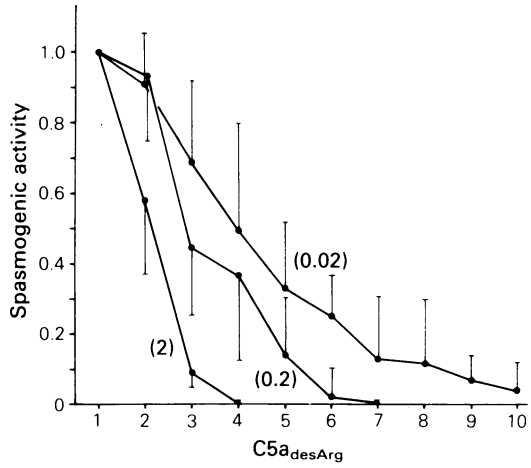

Figure 3 Concentration-dependence of deactivation to the spasmogenic action of $C5a_{desArg}$. The peptide was repeatedly (abscissa scale) applied in concentrations (in parentheses) of 0.02, 0.2 or 2 $\mu g\,ml^{-1}$ (contact times 30 s). $n = 5$; mean values with s.d. shown by vertical lines. Ordinate scale: spasmogenic effect of $C5a_{desArg}$ related to that of its 1st application (= 1.0). ACh was given in these experiments four times between the peptide applications, as $C5a_{desArg}$ at the highest concentration of 2 $\mu g\,ml^{-1}$ slightly reduced the effect of the next two ACh applications.

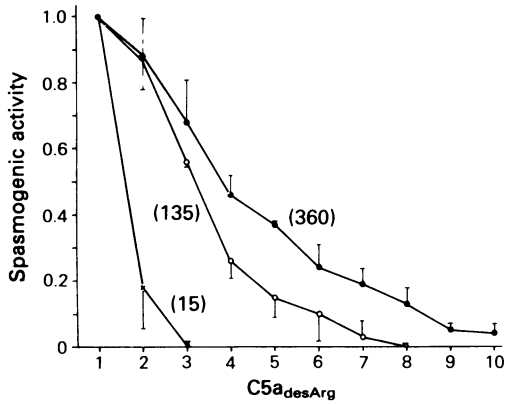

Figure 4 Influence of frequency of repeated $C5a_{desArg}$ applications (0.02 $\mu g\,ml^{-1}$, number of applications on the abscissa scale, contact times 30 s) on deactivation of guinea-pig isolated ileum segments. The frequencies are indicated by the time intervals (in s, see number in parentheses) between washing out of the previous and application of the following peptide dose (number of intermediate ACh applications were 0, 1 or 4 in the order of increasing time intervals). $n = 5$; mean values with s.d. shown by vertical lines. Ordinate scale: spasmogenic activity of $C5a_{desArg}$ relative to ACh response.

(4) *Influence of frequency of $C5a_{desArg}$ applications on deactivation*

The course of deactivation was influenced by the frequency of peptide applications: ileum segments repeatedly stimulated by 0.02 $\mu g\,ml^{-1}$ $C5a_{desArg}$ with a latency of 360 s (latency = time interval from washing out of the last concentration to application of the next dose) still responded weakly to the 10th application. At a latency of 135 s sensitivity was lost after the 7th application and at a latency of 15 s after the 2nd application (Figure 4).

However, a large proportion of sensitivity lost at the shortest latency of 15 s apparently recovered rapidly: at the 2nd application of $C5a_{desArg}$ the motor response was decreased to about 20% of the initial one, whereas with longer latencies (135 and 360 s) it was only slightly diminished (to 90%); this means that 70% of the responsiveness reappeared within 120 s. Therefore, besides the well-known slow deactivation (which, as will be shown, is followed by a slow recovery), an additional component with rapid onset and rapid reversal aggravates loss of sensitivity at short latencies (for details of this see Damerau *et al.*, 1985b).

The rate of deactivation was not affected by the number of intermediate ACh applications (tested at 210 s latencies with either one or four ACh stimuli,

data not shown). Furthermore, the accelerated loss of sensitivity at short latencies was not due to an unspecific decrease in sensitivity of the ileum segments, for example by a refractory period of the smooth muscle cells. ACh and histamine caused undiminished contractions when given at high frequencies.

(5) *Time course of recovery from slow deactivation*

Ileum segments which had been completely deactivated by one 10 min treatment with 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$ appeared to begin to respond to the same peptide concentration within a few minutes of incubation at 34°C in fresh Tyrode solution (Figure 5). Recovery followed an exponential time course. Curve fitting of the results of the individual experiments was performed according to the function $f(t) = a(1 - e^{bt})$; the mean value of 'a' which represents the maximum recovery was 0.54 ± 0.18, spasmogenic effect relative to maximal ACh response. The inverse relaxation time 'b' which indicates the progress of recovery appeared to be 0.035, this results in a mean relaxation time of 29 min; the individual values varied from 15 to 57 min. Furthermore,

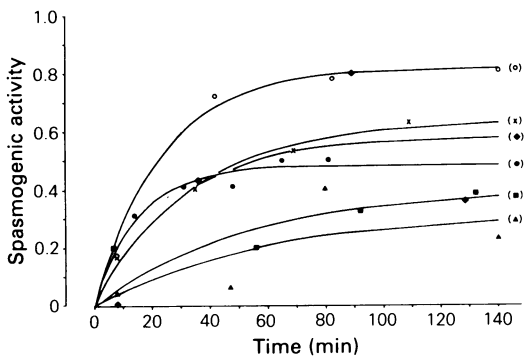

Figure 5 Time course of (slow) recovery of completely deactivated ileum segments to the spasmogenic effect of 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$. $n = 6$ (same symbols indicate ileum pieces from the same animal). Ileum segments of about 20 cm in length had been deactivated by 10 min incubation at 34 °C in Tyrode solution containing 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (this medium was renewed after 3.3 and 6.7 min) and were then incubated in peptide-free Tyrode solution. After different times 3–4 cm segments were cut, mounted in the organ bath, contracted three times by ACh and then tested with 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (abscissa scale: time for recovery in min, beginning with the incubation in peptide-free medium). Ordinate scale: spasmogenic effect of $C5a_{desArg}$ relative to ACh response. The curves for the individual experiments were obtained by non-linear least-square fitting as described in Methods.

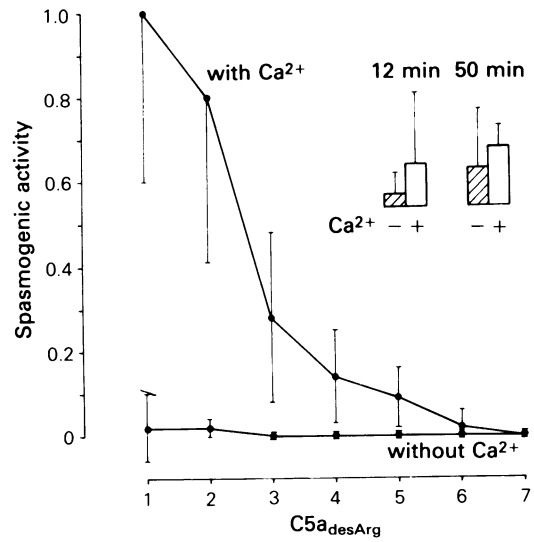

Figure 6 Lack of dependence of deactivation on the presence of Ca^{2+} ions in the medium. $n = 5$; mean values with s.d. shown by vertical lines. Ordinate scale: spasmogenic effect of repeated applications (abscissa scale) of 0.05 $\mu g\,ml^{-1}$ $C5a_{desArg}$ relative to ACh response (contact times of $C5a_{desArg}$ 60 s). During the initial test series the ileum segments were incubated either in Ca^{2+} – containing or Ca^{2+} -free Tyrode solution; thereafter all of them were transferred to Ca^{2+}-containing Tyrode solution and stimulated again 12 min and 50 min later by 0.05 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (contact times of 30 s). Hatched columns = reaction of ileum segments initially incubated in Ca^{2+}-free medium; open columns = reaction of test tissues initially kept in Ca^{2+}-containing Tyrode solution.

the curves in Figure 5 show that recovery usually reached its maximum after about 80 min. These data are approximate since the degree and course of recovery varied considerably between tissues from different animals (Figure 5).

When after recovery the ileum segments were deactivated again by repeated application of $C5a_{desArg}$ (normal test rhythm), about 2/3 of them were deactivated much faster than initially; in the other 1/3 the course of deactivation remained unchanged ($n = 50$).

(6) *Influence of Ca^{2+} ions in the medium on deactivation*

Except for the first two applications (which occasionally induced minimal contractions), $C5a_{desArg}$ did not contract ileum segments in Ca^{2+}-free Tyrode solution (lower curve, Figure 6). After subsequent incubation periods of 12 and 50 min in Ca^{2+}-containing

Tyrode solution they responded to ACh almost normally (70% of the controls, data not shown), whereas the reaction to $C5a_{desArg}$ was considerably reduced (Figure 6). This shows that deactivation depends neither on Ca^{2+} nor on the actual spasmogenic effects of $C5a_{desArg}$.

(7) Temperature dependence of mast cell degranulation, deactivation and recovery

Segments of ileum taken from the organ bath after treatment with ACh regularly showed some degranulated mast cells, about 14% on average. $C5a_{desArg}$ (0.08 and 0.16 $\mu g\,ml^{-1}$) considerably increased the proportion degranulated (72%) when in contact with the ileum at 34°C. At 16°C it had no effect on mast cells (13% degranulation; $n = 5$).

At 16°C $C5a_{desArg}$ also did not cause a contraction (lower curve, Figure 7), whereas agents directly stimulating smooth muscle cells such as ACh and histamine were still active. After 7 applications of $C5a_{desArg}$ at 16°C and subsequent rewarming to 34°C ileum pieces responded much less to the peptide than non-pretreated control tissues (2nd and 3rd column, Figure 7). Hence, deactivation but not contraction proceeded at 16°C, although to a lesser extent than at 34°C.

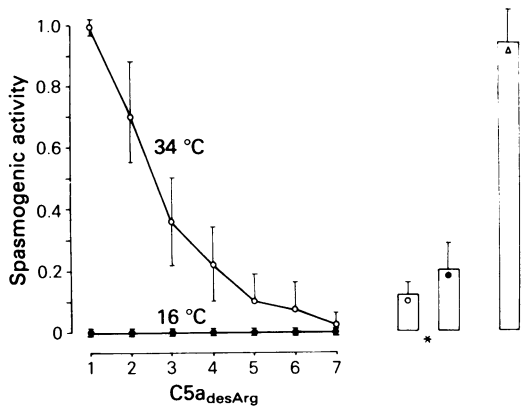

Figure 7 Deactivation of isolated ileum segments at 16°C and at 34°C by repeated applications (abscissa scale) of 0.04 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (contact times of 30 s). $n = 6$; mean values with s.d. shown by vertical lines. Ordinate scale: spasmogenic activity of $C5a_{desArg}$ relative to ACh response. Contact time of ACh given intermediately was 30 s (instead of 15 s as in the other experiments), because ACh-induced contractions were delayed at 16°C. After the 7th application of $C5a_{desArg}$ all ileum segments were incubated for 5 min at 16°C, then warmed up to 34°C and after 3 ACh applications tested with 0.06 $\mu g\,ml^{-1}$ $C5a_{desArg}$. Columns show reaction of ileum segments deactivated at 34°C (○), 16°C (●) and of control segments (▽) which had been treated with 10^{-7} M ACh only, for the same time at 16°C. *Statistically significant difference, $P < 0.05$.

However, when ileum segments had been incubated at 2°C for 20 min with a highly supramaximal concentration of $C5a_{desArg}$ (0.8 $\mu g\,ml^{-1}$) and were then extensively washed for 45 min at this temperature, they did not show any decrease in sensitivity to $C5a_{desArg}$ after the temperature had been increased to 34°C. On the other hand, if deactivation had already been induced (by incubation with 0.16 $\mu g\,ml^{-1}$ $C5a_{desArg}$ at 16°C for 4 × 3 min), it was not reversible during a subsequent washing period of 90 min at the low temperature (2°C) in peptide-free Tyrode solution.

Recovery from slow deactivation was suppressed at 16°C: after almost complete deactivation (at 34°C) and subsequent incubation at 16°C for 90 min followed by rewarming, ileum segments responded only minimally to 0.05 $\mu g\,ml^{-1}$ $C5a_{desArg}$ (0.07 ± 0.05 spasmogenic effect relative to maximal ACh response), whereas control segments which after deactivation had been incubated for 80 min at 34°C and then for 10 min at 16°C were contracted to a much higher degree (0.34 ± 0.2; $n = 6$). The inhibition of recovery at 16°C was overcome by raising the temperature to 34°C, but only when the organs were tested further with $C5a_{desArg}$ at sufficiently long test intervals (12 min); at shorter intervals (4 min) only minimal sensitivity could be regained.

Discussion

The course of slow deactivation of guinea-pig ileum segments towards $C5a_{desArg}$ is shown to be a highly reproducible process dependent on agonist concentration, frequency of stimulation and contact times. In experiments on the frequency-dependence, a fast and a slow component of deactivation could be differentiated. The fast component, characterized by rapid onset and reversal in about 3 min, is analysed in a subsequent paper (Damerau *et al.*, 1985b). It can be disregarded in the interpretation of results from the other experiments, because the latencies between peptide applications were longer than 3 min.

The slow deactivation by $C5a_{desArg}$ is apparently not due to exhaustion of histamine stores as has been proposed earlier (Hahn & Oberdorf, 1950; Rocha e Silva *et al.*, 1951). This conclusion, which has already been drawn by other authors (Friedberg *et al.*, 1964; Bodammer & Vogt, 1970) was confirmed by the following results: deactivation was induced under conditions (exposure to $C5a_{desArg}$ in Ca^{2+}-free medium or at 16°C) which prevent motor response and degranulation of mast cells. On the other hand,

the slow deactivation does probably concern the mast cells. Liberation of histamine has been shown to decline gradually on repeated application of $C5a_{desArg}$ (Bodammer & Vogt, 1970; Sorgenfrei *et al.*, 1982) indicating that they become increasingly unresponsive. However, that deactivation occurs equally in the smooth muscle which could become gradually refractory to a direct effect of $C5a_{desArg}$ cannot be excluded.

Compilation of a larger number of experiments ($n = 100$) resulted in an exactly exponential curve with a correlation coefficient of approximately 1.0. Hence, deactivation follows first-order kinetics, i.e. the extent of deactivation comprises a constant percentage of the momentary response. Furthermore, the course of deactivation strongly correlates with the cumulative contact times of $C5a_{desArg}$ with the test tissues and it is specific for this peptide. We propose that the slow deactivation is mainly due to occupation and subsequent block of specific binding sites on mast cells and possibly on other cells involved in the response. We also propose that under constant conditions (concentration, contact time) the same percentage of the receptors which are still free and available is occupied by each $C5a_{desArg}$ application and the rate and proportion of receptor occupation (on-rate) determines the strength of contraction. This is in accordance with studies comparing receptor binding of C5a or *N*-formylated oligopeptides and functional activity of leukocytes (Chenoweth & Hugli, 1978; Zigmond, 1981; Sklar *et al.*, 1982; Rossi *et al.*, 1983).

Deactivation of the ileum could not be diminished, either by extensive washing or by prolonged incubation (60 and 90 min) at 16°C and at 2°C. $C5a_{desArg}$ seems to bind to its receptors in gut quite strongly as has been found previously in leukocytes (Chenoweth & Hugli, 1978). On the other hand, the independence of deactivation from temperature variation (determined at 16°C and at 34°C) indicates that metabolic processes which induce some unspecified decay of receptor binding are not involved.

In leukocytes, internalization of peptide-receptor complexes and recycling of receptors have been demonstrated (Niedel *et al.*, 1979; Weinberg *et al.*, 1981; Chenoweth & Goodman, 1983). If these processes also occur in the ileal target cells of $C5a_{desArg}$, internalization should not contribute to deactivation to any great extent, since it develops at 16°C at which temperature internalization of e.g. insulin receptors is totally prevented (Marshall & Olefsky, 1981). Signal transfer and effector processes are also unlikely to contribute significantly, as they should also be inhibited at lower temperatures.

Deactivation appears to start instantly (at least within 15 s) and proceeds rapidly, its extent being doubled in 45–90 s when optimal peptide concentrations are applied. A similar time course was found for binding of complement peptides as well as *N*-formylated oligopeptides to leukocyte receptors (Chenoweth & Hugli, 1978; Sullivan & Zigmond, 1980; Rossi *et al.*, 1983), but our findings contrast with the lag period of about 45 s after which *N*-formylated oligopeptides begin to deactivate human neutrophils (Seligman *et al.*, 1982).

Between 16°C and 34°C deactivation/binding is little affected by temperature variation. However, at 2°C, deactivation could not be induced although $C5a_{desArg}$ did reach its target cells. The latter is indicated by the following results (not shown): ileum segments treated with $C5a_{desArg}$ at 2°C and then washed at this temperature for only 5 min, turned out to be completely deactivated when subsequently tested at 34°C. Obviously $C5a_{desArg}$ had reached its receptor, and the not yet fully washed out agonist then deactivated the tissue during the warming up. After a longer washing period of 40 min at 2°C the response remained normal.

It is possible that at 2°C the kinetic energy of the ligand molecules is too low for receptor binding at 2°C. On the other hand, deactivation caused by incubation with $C5a_{desArg}$ at 16°C was not reversed by incubation and washing at 2°C. Hence, the strength of binding is probably not decreased at 2°C.

After slow deactivation was complete, ileum strips gradually regained their sensitivity at 34 °C, but not at 16 °C. A weak motor response occurred within 5–20 min incubation; recovery reached its optimum (approximately 50% of the first response) after about 80 min, but the time course and the maximum recovery varied considerably. The fact that guinea-pig ileum strips recover after complete deactivation has been demonstrated by Friedberg *et al.* (1964) and by Bodammer & Vogt (1970), though the time course was not described by these authors. Curve fitting of our results led to an exponential curve. Receptor recovery as a logarithmic function of time has been found for receptors of *N*-formylated oligopeptides in rabbit polymorphonuclear leukocytes (Zigmond *et al.*, 1982).

The question arises as to whether the fast and slow components of deactivation are due to two types of C5a receptors with different affinities. This is unlikely, because the two components of deactivation show very different characteristics: in contrast to the slow component, the fast component is stimulus-unspecific, but effect- and possibly cyclicAMP-dependent (Damerau *et al.*, 1985b).

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