CHEMICAL TRANSMISSION AND ADAPTATION

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INTRODUCTION

The discovery of the stretch receptors of crustaceans (1, 2) and the recognition of their function by physiologists (3-6, 8, 12, 16) have opened new possibilities for the attack on neurophysiological problems of general interest. One becomes more and more convinced that the sensory neurons of these receptors can be considered as models of neurons in general. There is evidence that these neurons receive an efferent regulatory innervation and thus it becomes feasible to study general problems of synaptic transmission in stretch receptor preparations.

According to histological studies (9) the stretch receptor neurons of the crayfish receive endings of at least one efferent fiber and there is a possibility that they are also innervated by a second fiber, just as is the case in the stretch receptors of lobsters (1). The findings of Kuffler and Eyzaguirre (13) show conclusively that there is one efferent fiber which inhibits the discharge of the sensory stretch receptor neurons of the crayfish. If there is another efferent supply this might be excitatory (1, 9, 13).

Wiersma, Furshpan, and Florey (16) have described the excitatory effect of acetylcholine in these receptors and its potentiation by eserine as well as its block by atropine. Further pharmacological studies have shown that the receptor neurons are specifically sensitive to acetylcholine and one is tempted to assume that acetylcholine is the transmitter substance of a regulatory excitatory fiber as is the case in the heart ganglia of the crayfish (17). Actually this assumption is based on the following points: (a) The rather specific effect of acetylcholine (5) points to the existence of a specific acetylcholine receptor substance on the neuron. (b) The potentiating effect of eserine indicates the presence of choline esterase which is usually connected with cholinergic transmission. (c) Atropine does not block the response of the neurons to stretch but blocks applied acetylcholine (16). It would therefore seem unlikely that acetylcholine is involved in the transmission of excitation from stretched muscle to neuron. It is, however, quite likely that acetyl-

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choline is normally involved in the transmission of excitation from regulatory nerve endings to the sensory neuron (a view already expressed by Wiersma, Furshpan, and Florey (16)).

It has been shown that the stretch receptor neurons also respond to an inhibitory substance, factor I (5). There are strong arguments in favor of the view that the active principle of factor I is a transmitter substance of inhibitory neurons in crustaceans as well as in mammals (5, 7, 10, 11). In the stretch receptor neurons factor I blocks or diminishes the response to stretch and counteracts the action of acetylcholine.

So far we feel justified in assuming that acetylcholine and factor I are the transmitter substances of regulatory fibers whose endings make synaptic contact with the dendrites (and cell body?) of the stretch receptor neurons (9).

The response of the "slow adapting" neuron of crayfish stretch receptors to sudden increase or decrease of stretch has been described in a recent publication (8). It could be shown that a sudden increase in the length of the receptor muscle fibers is followed by an immediate jump in the impulse frequency which is followed by adaptation, consisting of a gradual decline of frequency until asymptotically a steady state is reached. Sudden relaxation of the muscle is accompanied by an immediate fall in discharge frequency (silent period) followed by a gradual return of impulses until a steady state frequency is reached. Adaptation was thus shown to take place in two directions and has been considered as an active process that leads to a return of the displaced membrane potential to a level close to the previous resting potential (which itself was the result of adaptation!). Adaptation can actually be directly compared with the well known process of accommodation in nerve fibers.

Method

Crayfish of the species *Cambarus virilis* Hagen were used as experimental animals. The stretch receptors were dissected and set up in the manner described previously (5). The dorsal half-shell of the "tail" served as a receptacle for the test solutions. It was freed from muscles except for the most lateral strand of the musculus superficialis lateralis (Schmidt (14)) which was left in place in that segment in which the stretch receptor organ was prepared. The nerve trunk containing the stretch receptor neurons was left attached to this muscle (Fig. 1). It was thus possible to prevent the nerve trunk from pulling on the delicate sensory nerve cells and their endings when the fluid was changed. Preparations in which changing of the fluid induced changes in the impulse frequency of the receptor neurons, were discarded.

Van Harreveld's salt solution (15) was used as saline medium. The addition of sodium-bicarbonate was, however, omitted. This solution will be called "crayfish solution." Factor I has been prepared according to the methods described by Florey and McLennan (10) and Elliott and Florey (3), but fresh brain extracts were also used repeatedly. The latter were prepared by boiling and homogenizing fresh or frozen

beef or rat brain in a tenfold (weight) amount of K-free crayfish solution. Acetylcholine as well as purified factor I was dissolved in crayfish solution.

The activity of factor I and its dilutions will be expressed in crayfish units (C.U. (3)), the crayfish unit being defined as the amount of factor I/ml. which is able to block the discharge of the slow adapting stretch receptor neuron of the third abdominal segment of a specimen of the crayfish *Cambarus virilis* Hagen for 10 seconds. Since variations of the sensitivity of stretch receptor neurons to factor I occur, a standard preparation of factor I was assigned the activity of 100 crayfish units per ml. (accord-

FIG. 1. This diagram represents the stretch receptor preparation as used in the adaptation experiments. *RM*, the stretch receptor muscles with the sensory neurons attached; *M*, musculus superficialis lateralis which remains in the segment in order to keep the nerve trunk anchored. *E*, platinum electrode over which the nerve is laid.

ing to the average sensitivity of a great number of test preparations). Determinations of activity on individual test organs were corrected for deviations in the sensitivity of the particular stretch receptor preparation to the standard solution of factor I. The corrected (absolute) values for factor I were thus expressed in "crayfish units reference" (C.U.R. (3)). In previous publications (7, 10, 11) the activity of factor I has been expressed in brain equivalents (B.E.).

In all the experiments to be mentioned in this paper the fast adapting nerve cells of the stretch receptor preparations were either crushed with the tips of fine forceps or their impulses, if they appeared at all, were disregarded. The nerve which contains the sensory axons was hung over a thin platinum electrode. Nerve impulses were recorded with an A.C. preamplifier and a single beam oscilloscope. An electronic counter together with a timing unit was used to determine the impulse frequencies at given intervals:

Usually three second counts were used, at intervals of 0, 15, 30, 60, and 90 seconds after application of saline or "transmitter"-solution.

RESULTS

(a) *Acetylcholine*.—The stretch receptor neurons of *Cambarus virilis* Hagen are not quite so sensitive to acetylcholine as are those of *Cambarus clarkii* Girard (16). However, concentrations down to $1 \mu\text{g}/\text{ml}$. are usually effective

FIG. 2. Response of one slow adapting stretch receptor neuron to applied and maintained acetylcholine of various concentrations. The drug was applied at the arrows and the vertical lines at the end of each sample curve represent the increase of the steady state frequency over the original frequency level.

in producing a marked increase in the impulse frequency of the sensory neuron. This excitation induced by acetylcholine follows a characteristic pattern: immediately after the application of acetylcholine the frequency jumps to a peak value whereupon it declines again, rapidly at first and then more slowly until a steady state is reached. Fig. 2 gives typical examples of several measurements taken from the same nerve cell. The values show a certain variation, but the pattern is obvious: after a sudden rise the frequency declines,

following an exponential curve which asymptotically approaches a steady state value. As shown in this figure, the sudden rise as well as the final steady state is proportional to the concentration of acetylcholine applied. There

FIG. 3. Responses of one slow adapting stretch receptor neuron to sudden increases and decreases in stretch of the receptor muscles. Note the difference in adaptation time with different amounts of relaxation of the muscles. The muscles were stretched by bending the abdominal half shell. The changes of length of the receptor muscles were estimated, using a micrometer eyepiece on the dissecting microscope.

is, however, no marked difference in the time required to reach the steady state (the "adaptation time") with different concentrations of acetylcholine.

The response of the sensory neuron to acetylcholine is obviously similar to the response to an increase in stretch and there seems to be no objection to the use in both cases of the term *adaptation* with respect to the fact that the maximum response is followed by a returning of the impulse frequency

to a level which is closer to the original frequency. To illustrate this statement Fig. 3 gives examples of measurements taken from the same nerve cell as the one used in Fig. 2, during stretch experiments. A more detailed analysis of the response of stretch receptor nerve cells to sudden changes in the length of the receptor muscle fibers has already been published (8). In this previous study it could be shown that the amount of increase of stretch does not alter the adaptation time although the amount of the immediate rise of the frequency and the final steady state are proportional to the increased stretch.

Since the adaptation of the sensory neuron follows an asymptotic curve no definite endpoint of the adaptation can be determined unless we specify a certain minimum rate of frequency change as arbitrary endpoint. For our purposes a minimum change of 0.25 per cent/sec. seems to be adequate. We

TABLE I

Concentration of acetylcholine applied	No. of cases in which a steady state of the impulse frequency was reached in					
	15-30 sec.	30-60 sec.	60-90 sec.	90-120 sec.	120-150 sec.	150-180 sec.
10^{-6}	11	23	26	17	3	
5×10^{-6}		2	18	36	8	
10^{-5}			11	48	7	
2×10^{-5}			12	62	9	
4×10^{-5}			13	65	11	2
6×10^{-5}			13	71	15	1
10^{-4}			4	75	25	3

The values presented in this table were obtained from seventeen stretch receptor preparations.

can now state that the adaptation is accomplished in approximately 2 minutes, whether the preceding stimulus was an increase in stretch or the application of acetylcholine. Representative values are given in Table I.

If the acetylcholine is now withdrawn and replaced by plain saline medium, the frequency decreases instantly to a lower level but immediately it begins to rise again, fast at first and then more slowly until it reaches the original value, that is the impulse frequency before the application of acetylcholine. The length of the silent period which follows the withdrawal of acetylcholine is proportional to the amount of acetylcholine that had been applied. Fig. 4 gives several examples. It becomes obvious that the adaptation time here *depends* on the concentration of acetylcholine removed.

Again, the situation is strikingly similar to that of a stretch receptor preparation whose muscle fibers are relaxed by varying amounts (see Fig. 3). In both cases the adaptation time increases with the amount of stimulus (stretch or acetylcholine) removed.

It is now interesting to compare the effect of acetylcholine on a stretch

receptor preparation whose muscle fibers were somewhat more stretched so that the steady state impulse frequency was raised. Although the variability of the responses was great it can be said that a preparation with a high rate of firing responds to acetylcholine by giving about the same number of additional impulses per second as a "low frequency" preparation. Table II gives representative values. As can be seen from this table the effect of acetylcholine is strictly additive.

FIG. 4. Inhibition induced by removal of various concentrations of acetylcholine (see Fig. 2). The drug was removed and replaced by plain saline medium at the arrows. The vertical lines at the beginning of each curve represent the increase of the steady state frequency previously effected by acetylcholine. Note the differences in adaptation time.

If acetylcholine was removed from a preparation with a high impulse frequency the silent period was shorter than if it was removed from a preparation that was firing at a low rate. The difference, however, becomes obvious only if the original impulse frequencies approach the extremes of the lowest or highest steady state values. Medium differences in the frequency level seem to be without influence on the silent period.

(b) *Factor I*.—The response of a stretch receptor neuron to a solution of factor I is in every detail the opposite of that to a solution of acetylcholine. There is an immediate drop in frequency but if the concentration of factor I

is low enough, the impulse frequency rises again until it reaches asymptotically a steady state value which is somewhat lower than the original frequency level. Concentrations of factor I higher than 0.5 C.U.R./ml. usually cause a silent period whose duration is determined by the concentration of factor I

TABLE II

Original frequency	Steady state frequencies after application of acetylcholine 2×10^{-4}	Average	Average increase due to acetylcholine
<i>impulses/sec.</i>	<i>impulses/sec.</i>		
5	14, 14, 15, 15, 16, 17, 18	15.5	10.5
10	14, 19, 21, 22, 22, 25, 26	21.2	11.2
15	24, 25, 25, 27, 29, 29, 29	26.8	11.8
20	30, 30, 30, 32, 32, 34, 34, 36	32.2	12.2
25	30, 35, 35, 35, 37, 41, 41, 41	36.4	11.4
35	42, 43, 45, 46, 49, 50, 51, 55	47.6	12.6
50	58, 58, 59, 62, 66, 66, 67	62.2	12.2

The values presented in this table were obtained from one individual neuron, using repeated application of acetylcholine. The "original frequency" is the steady state frequency after the neuron has adapted to the normal saline medium. It has been adjusted to various levels by changing the tension of the receptor muscle fibers.

TABLE III

Concentration of factor I	Length of silent period, sec.
<i>C.U./ml.</i>	
0.6	0, 0, 0, 0, 0, 1, 1, 2, 2
0.8	1, 1, 2, 2, 2, 2, 3, 3, 4
0.9	2, 4, 4, 4, 5, 6, 6, 6, 7
1.0	8, 8, 10, 10, 10, 10, 11, 11, 12
1.1	15, 15, 15, 16, 16, 17, 18, 18, 18
1.2	25, 26, 27, 27, 29, 35, 36, 37, 60
1.3	29, 39, 46, 48, 65, 77, 120, 120, 120

The values presented in this table were obtained from one individual stretch receptor neuron. The sequence of the different concentrations applied was varied in order to exclude effects of possible changes in sensitivity of the neuron as far as possible. The maximal time of contact between the neuron and factor I solutions was 120 seconds.

as well as by the sensitivity of the receptors to this factor. Table III gives representative values for the correlation between concentration of factor I and length of silent period.

Immediately after the replacement of the factor I solution by plain crayfish solution the frequency jumps to a peak value far above the original frequency and then decreases at once to a steady state level which is more or less equal to the steady state frequency before the application of factor I.

This effect can be seen in Fig. 5. Thus the removal of factor I has an effect similar to that of application of acetylcholine or of a sudden increase in stretch.

If the tension of the receptor muscle fibers was increased so that the resulting steady state frequency was about 20/sec., the silent periods were shorter and the course of adaptation was more rapid than in less stretched prepara-

FIG. 5. Responses of one slow adapting stretch receptor neuron to application and removal of various concentrations (expressed in C.U. = crayfish units (per ml.)) of factor I.

tions with frequencies of about 5 impulses per second. The excitatory effect of removal of factor I is, however, quantitatively the same whether it occurs in a stretched or a relaxed preparation. It is purely additive and follows the same time course regardless of the frequency level of the nerve cell and depends only on the concentration of factor I removed.

(c) *Stretch Responses in the Presence of "Transmitters."*—If during the excitatory action of acetylcholine the tension of the receptor muscles is lessened, there occurs a sudden fall in the frequency which is followed by a return to a steady state frequency which is lower than that originally induced by acetylcholine. If during the action of acetylcholine the muscles of the receptor organ are stretched, there occurs a sharp rise in frequency and an

asymptotic return to a steady state level somewhat above the acetylcholine-induced frequency.

The opposite is true for factor I. In its presence the preparation behaves as if relaxed. If the concentration of factor I is sufficiently low (e.g. 1.5 C.U.) but still blocking the discharge indefinitely, sudden increase in the stretch of the muscle fibers will cause the appearance of nerve impulses of moderate

FIG. 6. Responses of a slow adapting stretch receptor neuron to stretch or relaxation of its muscle fibers while treated with acetylcholine or factor I. 0, washing with saline medium.

frequency or even high frequency, but at once this frequency will decline and return to zero or a low frequency which then is determined by the interaction of stretch and factor I.

Examples of this behavior are shown in Fig. 6.

DISCUSSION

The present as well as previous (8) findings demonstrate that receptor neurons adapt not only to excitatory but also to inhibitory stimulation and that these neurons respond in this way not only to the stimulus (whatever its nature may be) but also to the removal of the stimulus. Removal of an excitatory stimulus to which a nerve cell has adapted, causes the same response as

an inhibitory stimulus and removal of the inhibitory stimulus is followed by a response identical with that of an excitatory stimulation.

This behavior cannot be found if the stimulus acts only briefly. It has been shown (8) that if the receptor muscle fibers are suddenly stretched and immediately relaxed to the original tension, the impulse frequency instantly rises to a high value (corresponding to the stretch applied) but returns without delay to its original level as the tension decreases; *there is no inhibition of the discharge*. Only if the increased stretch is maintained sufficiently long to permit adaptation will inhibition occur as the stretch is released. The same (with reversed signs) is true for sudden relaxation and immediate restretching. The adaptation process thus has a much greater inertia than the immediate response of the neuron. Frequency changes elicited by excitatory or inhibitory stimulation (whatever the nature of the stimulus may be) are therefore the resultant of the differential between the inertia of the immediate response and that of adaptation (even if adaptation were understood only as the exponential factor of decay of the immediate response). This in turn means that the response of the neuron to excitatory or inhibitory stimulation will depend on the rate of change of those environmental factors which, due to their changing, provide the stimulus. In the extreme case, that is when the rate of change equals the time course of the adaptation process, the immediate response should be identical with the "steady state." This situation is comparable to the *Einschleich* phenomenon of the nerve fiber, in which there is no conducted response if the time course of the rising stimulating current equals the time course of accommodation.

It has been noted on several occasions in this and the previous paper (8) that the adaptation time seems to be rather independent of the size of the stimulus if this is excitatory, but that it is proportional to the stimulus if the stimulus is inhibitory in nature. Obviously there must be two different mechanisms at work. This is the more probable as it seems logical to assume that an increase in the discharge rate is caused by a different factor than the slowing of this rate.

We know that stretch depolarizes nerve endings and cell body of the stretch receptor neuron and that the frequency of firing of the neuron depends (within limits) on the level of the membrane potential. This has already been extensively discussed (6, 8, 12, 13). Acetylcholine is known to be a depolarizing substance and we may assume that factor I has repolarizing properties.¹ The impulse frequency could then be taken as a measure of the membrane potential. Adaptation, as measured by the impulse frequency, would thus also be a phenomenon of the membrane potential. The processes involved in

¹ This is presently being studied by means of intracellular microelectrodes, in collaboration with Dr. Robert Martin.

adaptation might therefore be those which are involved in the maintenance and change of the membrane potential of nerve cells. It seems now to be of considerable interest to study the effect of variations in the ionic composition of the external saline medium on adaptation.

Whatever the mechanism of adaptation may be, it seems important that a neuron has been found to adapt to the action of those chemicals which are considered to be transmitter substances normally active on this nerve cell. This means that a neuron is able to shut itself off from the action of a released transmitter, even if this transmitter is not removed by circulation or enzymatic action. It seems interesting to speculate along the line that even the neurons in the central nervous system or in autonomic ganglia can be considered as "sensory" or receptor neurons inasmuch as they respond to chemical stimuli, that is to transmitter substances. We should therefore expect to find adaptation also taking place in central neurons. Phenomena such as post-excitatory depression or postinhibitory excitation would thus find an obvious explanation.

The findings reported in this paper perhaps open a new perspective for the study of drug actions: it is very well conceivable that neurogenic drugs act not only by replacing or blocking transmitter substances, inhibiting antitransmitter enzymes or directly affecting membrane potentials, but they might very well affect the mechanism of the adaptation process. A study of the action of drugs on the time course of adaptation seems, from this point of view, promising.

SUMMARY

Acetylcholine and factor I appear to be transmitter substances of excitatory and inhibitory regulatory nerve fibers supplying the sensory neurons of stretch receptor organs of the crayfish.

Sudden application of a low concentration of acetylcholine causes the impulse frequency to jump to a peak value. But immediately the frequency falls again and gradually reaches a steady state which is not far above the previous frequency level. If the acetylcholine is now withdrawn there follows a silent period after which the frequency returns to its original level. The time course of these events is identical with that of adaptations to sudden increase or decrease of stretch.

Factor I in sufficiently low concentrations causes an immediate fall in impulse frequency (silent period) which is followed by a return to a value near the previous frequency level. Withdrawal of factor I is followed by excitation and again return of the frequency to the rate measured before the application of factor I. The time course of these phenomena is identical with that of adaptations to sudden decrease and increase of stretch.

It is suggested that adaptation may be a property not only of sensory

neurons but of neurons in general and that even central neurons may be considered as receptor neurons inasmuch as they respond to chemically transmitted excitatory and inhibitory stimuli.

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