Quantal Mechanism of Transmitter Release during Progressive Depletion of the Presynaptic Stores at a Ganglionic Synapse

The action of hemicholinium-3 and thiamine deprivation

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ABSTRACT In the present experiments we interfered with the mechanism of acetylcholine (ACh) synthesis in the rat superior cervical ganglion by impairing the supply of either the choline group (hemicholinium no. 3 [HC-3] treatment) or the acetyl group (thiamine deprivation). Under both conditions stimulation causes in the ganglion a progressive decline in ACh output associated with a depletion of transmitter tissue content. ACh release from the terminals of a single preganglionic fiber was estimated from the quantum content value of the evoked excitatory postsynaptic potentials (EPSP's) recorded intracellularly in the ganglion neuron under test. The present observations indicate that Poisson statistics describe transmitter release at either low or high release levels. Furthermore, the progressive decline in the rate of ACh output occurring during repetitive stimulation is shown to correspond to a progressive decrease in the number of transmitter quanta released per impulse and not to any modification in the size of individual quanta. Some 8,000 transmitter quanta proved to represent the presynaptic transmitter store initially present in those terminals on a neuron that are activated by stimulation of a single preganglionic fiber. Speculations are considered about synaptic efficacy and nerve connections in rat autonomic ganglia. It is suggested that six preganglionic fibers represent the mean input to a ganglion neuron.

INTRODUCTION

The mammalian superior cervical ganglion is a convenient preparation for the study of synaptic transmission with regard to the quantal mechanism by which transmitter release is brought about (33). Despite the presence of multiple and distributed innervation upon the sympathetic neurons it has
been possible to accumulate considerable information concerning the quantal nature of the synaptic responses, and the conventional statistical methods for evaluating presynaptic activity, usually applied at the neuromuscular junction (17), also proved to be successfully applicable at the ganglion synapse (3, 20, 27, 33). This provides a favorable approach for quantitative analysis of the alterations in synaptic activity under different experimental conditions.

There is now no doubt that acetylcholine (ACh) is the neurotransmitter at the interneuronal synapse of sympathetic ganglia, but details of its synthesis and release are still incomplete. Choline and acetyl-CoA are obligatory precursors of ACh in a reaction catalyzed in the nerve terminals by choline acetyltransferase.

Hemicholinium no. 3 (HC-3) is frequently used in studies on the uptake of choline by nerve membranes and its further acetylation to ACh. It is well known that this drug competitively inhibits the uptake of choline (25, 31) and hence interferes with the synthesis of ACh necessary to maintain normal tissue levels (2, 23, 32).

Several substances have been found capable of functioning as acetyl donors for ACh synthesis. Pyruvate is however the most important precursor of the acetyl moiety of ACh in the brain (6, 37). Acetyl-CoA is formed from pyruvate by an oxidative decarboxylation catalyzed by pyruvate dehydrogenase, a thiamine-dependent enzyme. Thiamine deficiency has been studied in this investigation in view of the role played by thiamine diphosphate in the production of acetyl-CoA from pyruvate. It was felt in fact that thiamine deprivation was a unique means of interfering with the synthesis of the acetyl moiety of ACh. Available information supports the view that ACh synthesis is linked to the pyruvate → acetyl-CoA pathway and the action of thiamine. The concentration of ACh in the brain and spinal cord of thiamine-depleted rats was found to be decreased (7, 22, however 14, 36). Furthermore, Mann and Quastel (24) have demonstrated that the rate of synthesis of ACh in the brain in vitro is much slower in thiamine-deficient than in normal pigeons. The addition of thiamine restored the capacity to synthesize ACh in polyneuritic brains but failed to influence normal brains. Finally a severe impairment in synaptic transmission has been shown to occur in the superior cervical ganglion of the thiamine-deficient rat, in which the ability to transmit nervous impulses at high frequency is dramatically lost (28).

Experiments using cat sympathetic ganglia perfused with HC-3 have demonstrated that repetitive preganglionic stimulation causes a progressive decline in the rate of ACh release and a parallel decrease in the tissue content of ACh (2). A similar depletion of 70% in ACh tissue levels has been observed in the superior cervical ganglion excised from thiamine-deficient rats as a result of a 30 min preganglionic stimulation period at 10/s (H. Ladinsky, S. Consolo, O. Sacchi, and V. Perri, in preparation). Under both conditions,
i.e. HC-3 treatment and thiamine deprivation, the stores of transmitter present in the nerve terminals before the beginning of stimulation are depleted. The purpose of this study was to investigate the synaptic mechanism whereby these stores are exhausted.

Preliminary reports on some of the findings have already been briefly presented (34, 35).

METHODS

Isolated superior cervical ganglia excised under ethyl urethane anesthesia from normal and thiamine-deficient rats (Wistar strain) were used throughout. The preparations were placed in a Perspex cell and superfused at 37°C with continuously flowing saline into which a mixture of 95% O2 and 5% CO2 was bubbled. The bathing solution had the following ionic composition (millimolar): NaCl 136, KCl 5.6, CaCl2 2.2, MgCl2 1.2, NaH2PO4 1.2, NaHCO3 14.3, glucose 5.5. Choline chloride 3 x 10^-5 M was added in all cases, except when HC-3 was used. Ca2+ and Mg2+ concentrations were maintained unchanged in all the experiments. Intracellular recordings were obtained by conventional glass microelectrodes filled with 2 M K-citrate, having high tip resistances (30-60 MΩ, measured in the bathing fluid). Further details in techniques for nerve stimulation and intracellular recording of synaptic responses have been fully reported elsewhere (28, 29).

EPSP's were recorded from ganglion neurons during prolonged repetitive stimulation of an afferent fiber at 1/s and 10/s. The mean quantal content of the evoked synaptic responses was calculated from the percentage of failures (m1), the ratio of mean EPSP amplitude to mean amplitude of the spontaneous potentials (m1) and the coefficient of variation of EPSP amplitude (m2). The estimated values were derived from samples of about 200 EPSP's in the series at 10/s and 60-100 EPSP's in those at 1/s. The definitive evaluation of quantum content was preferably given as \( m = (m_1^2 - m_2) / 1 \), this estimate being unaffected by nonlinearity of the postsynaptic response and independent of driving potential amplitude for the transmitter (22, 23). This appears to be relevant, since synapses in mammalian sympathetic ganglia are known to be mainly axo-dendritic in nature; in this case the unit potentials at the synaptic site may be as large as 15 mV (21) and it will be difficult to make the appropriate correction for their nonlinear summation. The estimate of quantum content from \( m_1 \) is also unaffected by nonlinear summation, but becomes inaccurate as quantum content increases and unreliable when greater than 4 (9). In a few experiments in which the paucity or absence of spontaneous potentials ruled out any possibility of evaluating \( m_1 \), analysis was limited to the application of the variance method and the mean quantal content given as \( m_2' \), after correction for nonlinearity of the postsynaptic response (22).

An error resulting in an underestimate of \( m_2 \) by the variance method may arise owing to drift in the mean amplitude of the evoked EPSP's occurring slowly during repetitive stimulation (5, 8). Linear regression of EPSP amplitude over a period of 20 s was not however statistically significant in most of the experiments (Fig. 1 B-D). The system was therefore considered to be in a steady state during each of the 20-s intervals necessary to collect the 200 EPSP samples used to evaluate \( m_2 \). Moreover, the defini-
tive estimate of quantum content depends in the analyses not only on $m_2$ but also on $m_1$, which is clearly unaffected by regression of EPSP amplitude. Fig. 1A shows a train of evoked EPSP's in which, unusually, the linear regression over time was significant. The evaluation of quantum content by the variance method without subtracting the variance due to regression resulted in an underestimate of $m_2$ in this series by 2.2%, which was further reduced to 0.7% when quantum content was given as $m$. The error was sufficiently small to be ignored.

The quantal size of the unit potentials building up the evoked EPSP's in a series was evaluated both directly from the mean amplitude of the miniature potentials occurring in that series and from the ratio of mean EPSP amplitude to the corresponding quantum content value. Mean EPSP amplitude ($\bar{V}$) was preliminarily corrected for nonlinear summation of the unit potentials according to the formula (13):

$$\bar{V} = \frac{\bar{V}}{1 - \bar{V}/V_o} + \frac{\text{var } V}{(1 - \bar{V}/V_o)} 

$$

where \text{var } V is the variance of EPSP amplitude distribution and $V_o$ the driving potential for ACh, an EPSP equilibrium potential of $-10$ mV being assumed.

The thiamine-deprived rats were kept on a thiamine-deficient diet for 28–32 days.
and were killed as soon as signs of neurologic dysfunction became apparent. Hemicholinium no. 3 was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.

RESULTS

Stimulation of a single afferent fiber was a necessary prerequisite for obtaining subthreshold activation of the ganglion neuron and EPSP's arising from a single synapse or from neighboring synapses, as indicated by similarity in rise time, shape, and time-course of the postsynaptic responses (33). The finding, furthermore, that the onset of indirect stimulation noticeably increases the frequency of spontaneous potentials, which otherwise are virtually absent at rest, makes it possible to evaluate correctly the size of the basic units building up the evoked EPSP's, miniature potentials being originated only from the terminals activated by stimulation (33). Under these conditions the statistical treatment of EPSP amplitude fluctuations can be successfully undertaken, since one can safely assume that EPSP quantal components are the same size as the miniature potentials and are not differently attenuated after different dendritic spreading.

Despite the presence of multiple innervation upon the sympathetic ganglion neurons, focal synaptic activation of the cell surface was obtained by evoking the EPSP's via an axon reflex elicited by stimulating the internal carotid nerve, which is a postganglionic trunk containing preganglionic through-fibers (30). The degree of convergence of this particular type of fiber on single neurons is very low. In some experiments, in fact, the presence of a single input on the neuron under test was demonstrated by the absence of recruitment in the presynaptic input irrespective of the stimulus strength. In other cases the progressive recruitment of afferent fibers in increasing steps was controlled by grading stimulus intensity. Activation of a single preganglionic fiber of low threshold running in the sympathetic trunk was also obtained in some preparations after appropriate adjustment of the stimulus (3, 33).

Preganglionic Stimulation in Normal Preparations

Fig. 2 shows the amplitude fluctuations of the EPSP's recorded from a neuron of rat superior cervical ganglion superfused with normal saline at various times during prolonged stimulation of a preganglionic fiber running in the internal carotid nerve. The high degree of amplitude fluctuation in sequential EPSP's reveals their relatively low quantum content. The first EPSP in the first row of the figure is the very first EPSP of the train and it will be noted that there is no evidence at this stimulation rate of the "early tetanic rundown" occurring at the neuromuscular junction (see also Figs. 1 A and 4). On shifting stimulation frequency to 10/s a new level of transmitter release is immediately reached and further stimuli elicit EPSP's which are much
the same size as the first in the train, once allowance is made for the fluctuations inherent in the quantal nature of the synaptic responses. Transmitter release, as may be seen from the mean amplitude of the EPSP's in the different rows, is well maintained in this experiment for up to 12 min during the 10/s stimulation period.

It is possible to derive the basic parameters for a quantitative evaluation of the synaptic activity from this type of recording. Fig. 3 represents the time-course of changes in quantum content and mean EPSP amplitude, corrected for nonlinear summation of the postsynaptic response, in the experiment partially illustrated in Fig. 2. It is evident that modifications in mean EPSP amplitude are accompanied by parallel modifications in quantum content values. At different times during repetitive stimulation, quantum size was evaluated both directly from the mean amplitude of the spontaneous potentials and from the ratio of mean EPSP amplitude to the corresponding quantum content value. Both estimates were found to be in good mutual agreement and appeared unaffected by preganglionic stimulation throughout the whole observation period.

The results of five experiments from superior cervical ganglia superfused with normal saline are listed in Table I. Despite some differences between individual cells, the general pattern was fairly constant. The mean number of quanta delivered to a single neuron after stimulation of an afferent fiber at 1/s was found to be 3.96. An average decrease of 14% in the quantum content value was observed when the stimulation rate increased from 1/s to 10/s. After this, only minor fluctuations were observed as stimulation was
Figure 3. Time-course of changes in mean EPSP amplitude corrected for nonlinear summation (○), quantum content evaluated from m (■), mean amplitude of spontaneous miniature potentials (■), and quantum size calculated from the ratio of mean EPSP amplitude to m (▲) during prolonged stimulation at 1/s and 10/s of an afferent fiber. Data derived from EPSPs recorded in a ganglion superfused with normal saline.

Table 1

| Cell no. | Stimulation rate 1/s | Minutes after starting 10/s stimulation |
|----------|----------------------|---------------------------------------|
|          | 0  | 1  | 2  | 3  | 4  | 6  | 8  | 10 | 12 |
| 1        | 3.89 | 5.70 | 3.94 | 3.34 | 4.12 | 4.31 | 3.77 | 3.49 | 3.37 |
| 2        | 3.63 | 2.52 | 2.61 | 1.81 | 3.09 | 2.48 | 2.54 |      |    |
| 3        | 4.45 | 1.96 | 1.84 | 1.93 | 1.47 | 2.22 | 3.24 | 3.58 | 3.20 |
| 4        | 6.16 | 4.83 | 4.68 | 5.40 | 5.22 | 4.68 | 4.75 | 5.13 | 6.35 | 7.00 |
| 5        | 1.67 | 2.01 | 1.17 | 3.15 | 3.55 | 3.76 | 3.29 | 2.96 |      |    |

Mean 3.96 3.40 2.85 3.13 3.49 3.49 3.52

All the data are derived from neurons superfused with normal saline at various times during repetitive stimulation of an afferent fiber at 1/s and 10/s.

* Quantum content is evaluated from \((m_1^2 - m_2^2)^{1/2}\) in experiments 1, 2, 4, and 5; from \(m_2\) in experiment 3.
prolonged. Because of the limited number of observations it is in fact questionable whether the transient decrease in mean quantum content value observed 1 min after 10/s stimulation was started has any functional significance.

**Preganglionic Stimulation in the Presence of HC-3**

Resting sympathetic ganglia contain a preformed store of ACh. When ganglia are repetitively stimulated ACh is efficiently released and newly synthesized. In the presence of HC-3, stimulation causes a progressive decline in ACh output associated with depletion of ganglionic stores and inhibition of ACh synthesis. Presumably, the decline in transmitter output may have a counterpart in the quantal emission from the nerve terminals. Fig. 4 shows EPSP amplitude fluctuations recorded from a ganglion neuron in the presence of HC-3 6 × 10^{-6} M. It can be clearly seen from these tracings that 10/s stimulation causes evoked EPSP amplitude to run down dramatically and transmitter release to cease almost completely within a few minutes. The number of failures increases as stimulation proceeds and after 3 min the size of the evoked synaptic responses has become very small, most of the EPSP's probably consisting of single units. The progressive decrease in transmitter output does not appear to be due to failure in impulse conduction in preganglionic fibers occurring at high frequency stimulation. Complete blockade in synaptic transmission was actually observed in the isolated guinea pig superior cervical ganglion during preganglionic stimulation at 20/s and was considered to be the result of a conduction block (33). In this case, however, long sequences of evoked EPSP's of apparently constant quantum content and long series of failures tended to occur in an all-or-none fashion intermittently.

![Figure 4](image-url)
in alternating cycles. The pattern of the evoked responses was completely different in the experiments reported here. The number of failures increased as mean EPSP amplitude decreased, their occurrence becoming very frequent only when most of the EPSP's were reduced to unit potentials. Furthermore, the rather good fit between \( m_0 \) and \( m \) (Fig. 7) indicates that the high number of zero responses is completely accounted for by the statistical properties of the release process.

In Fig. 5 quantum content, mean EPSP amplitude, and quantum size, derived from an experiment similar to that illustrated in Fig. 4, are plotted against stimulation time. It will be noted that the size of the unit potentials building up the synaptic responses remains unchanged despite the progressive decrease in EPSP amplitude, which appears to be completely accounted for by a parallel decrease in the \( m \) value.

Quantum size was evaluated systematically at various times during stimulation in the experiments performed in the presence of HC-3. This appears to be pertinent since the presynaptic effect of the drug at the neuromuscular junction is thought to consist of a progressive decrease in transmitter quantum size (10, 11, 16), whereas the quantal content of the end plate potentials is affected to a much lesser extent (10). If the release of a reduced number of ACh quanta of constant size is alone responsible for the decrease in EPSP
amplitude, quantum content and EPSP amplitude should be linearly related. Fig. 6 reports the results obtained in two experiments carried out in the presence of HC-3 $6 \times 10^{-6}$ M in which mean EPSP amplitude corrected for nonlinear summation of the unit potentials is plotted against the corresponding $m$ value. The straight line in both cells plotted between the two values indicates that quantum size is unaffected by stimulation at either high or low levels of transmitter release. In all the other experiments there was no indication of any change in quantum size occurring in the course of long-lasting stimulation.

In order to obtain definitive evidence that the decrease in amplitude of the evoked EPSP's in the presence of HC-3 is related exclusively to a drift in the probability for a nerve impulse to release transmitter quanta of unchanged size, statistical treatment of postsynaptic responses was undertaken according to Boyd and Martin (4). Fig. 7 illustrates a complete analysis carried out on data obtained from cell no. 6 during exposure to HC-3 and shows the fit between the evoked EPSP amplitude distribution and the expected Poisson distribution at various times during a 10/s stimulation period. It is evident that a progressive shift of EPSP amplitude occurs from the classes of high quantum content to those of low quantum content as stimulation is prolonged. After 7 min the evoked EPSP distribution perfectly fits that of the spontaneous potentials recorded throughout the whole experiment. The rather good fit
FIGURE 7. EPSP and spontaneous potential amplitude distribution derived from a ganglion neuron during repetitive stimulation at 10/s of a preganglionic fiber running in the internal carotid nerve. HC-3 6 × 10^-6 M was present. Amplitudes were individually corrected for nonlinear summation. Spontaneous potentials were compared at different times after stimulation was started, and once it was ascertained that their size showed no regression over time (Fig. 5), they were pooled to give the best estimate of unit potential amplitude. The miniature potentials corresponding to the black area on the top of the figure were considered multiquantal and discarded in calculating $m_1$. The smooth lines superimposed on the observed EPSP distributions show the expected Poisson curves calculated according to Boyd and Martin (4). Quantum content value estimated from $(m_1^2-m_3)^1$. 
between the observed and predicted EPSP amplitude distribution indicates that Poisson statistics describe transmitter release at low and high release levels and that the quantal components of the EPSP's are the same even when ACh release level is low after depletion of the presynaptic stores by stimulation.

The results of nine experiments performed in the presence of HC-3 6 × 10⁻⁶ M are listed in Table II. The general pattern previously described appears to be constant in every way even when the preganglionic stimulation rate is decreased to 5/s or when choline chloride 10⁻⁵ M is added to the bathing solution (cells no. 7 and 10). The time taken for EPSP's to level out completely appears however to be inversely related to the stimulation frequency. After exhaustive

| Cell no. | Stimulation rate 1/s | Minutes after starting high frequency stimulation | Stimulation rate 1/s |
|----------|----------------------|-----------------------------------------------|----------------------|
|          | 0 1 2 3 4 5 6 7 8 10 12 |                                               |                      |
| 6        | 4.24 4.40 4.21 3.94 3.54 2.87 2.17 1.55 0.82 | 1.20 |
| 7        | 6.18 4.33 3.05 2.19 1.96 1.29 1.10 0.78 0.51 | 1.29 |
| 8        | 8.69 5.33 3.27 1.57 0.99 0.50 | 2.05 |
| 9        | 5.63 4.92 3.15 1.33 0.72 0.35 | 1.81 |
| 10       | 3.64 4.73 3.17 3.16 2.22 0.90 | 2.17 |
| 11       | 11.30 9.48 6.83 4.26 1.93 0.85 | 3.75 |
| 12       | 10.07 7.19 2.61 0.87 |   |
| Mean     | 7.08 5.65 3.78 2.49 1.89 1.11 | 2.06 |
| 13       | 7.21 6.91 5.29 3.38 1.91 1.40 1.37 1.08 3.43 |   |
| 14       | 2.36 1.97 2.06 0.98 0.70 |   |

* Quantum content evaluated from \( m_1 \) in cells no. 9 and 10; from \( (m_1^2 - m_0) \) in all other experiments.
\footnote{5/s in cells no. 13 and 14; 10/s in all other experiments.}

stimulation, recovery in quantum content is incomplete, as shown by its low values when stimulation rate is switched back again to 1/s.

**Preganglionic Stimulation and Thiamine Deficiency**

Long-lasting preganglionic stimulation of thiamine-deficient and HC-3-treated ganglia results in both cases in the exhaustion of ACh tissue content. It was felt that the alteration of synaptic efficacy involved might be comparable. The effects of thiamine depletion upon the quantal process of transmitter release at the ganglionic synapse proved in fact to be similar to those previously described in the HC-3 experiments. Fig. 8 illustrates the time-course of modifications in quantum content and EPSP amplitude occurring during 1/s and 10/s stimulation of an afferent fiber in a thiamine-depleted preparation. After a transient facilitation, the EPSP's decline in amplitude, the
change being invariably due to a decrease in quantum content while the quantal size of the unit potential remains constant. When stimulation was shifted back to the control frequency, EPSP amplitude and quantum content reverted to their full size within 2 min. ACh synthesis is in fact presumably impaired to a lesser extent in the thiamine-deficient ganglion than after HC-3 treatment, and this allows faster recovery in the rate of transmitter release when stimulation is reduced from a high frequency to 1/s (Tables II and III).

The relationship between mean EPSP amplitude and the corresponding quantal content was also carefully investigated in these experiments and no

![Figure 8](image_url)

**Figure 8.** Mean EPSP amplitude corrected for nonlinear summation (○), quantum content evaluated from \( m (\bullet) \), mean amplitude of spontaneous potentials (■), and quantum size calculated from the ratio of mean EPSP amplitude to \( m_0 (\triangle) \) plotted against stimulation time. Data derived from a thiamine-depleted preparation, in which a preganglionic fiber was activated at 1/s and 10/s.

| Table III | EFFECT OF REPETITIVE STIMULATION ON MEAN QUANTAL CONTENT* OF EPSP's FROM NEURONS IN THIAMINE-DEPLETED GANGLIA |
|-----------|---------------------------------------------------------------------------------------------------------|
| Cell no.  | Stimulation rate 1/s | Minutes after starting 10/s stimulation | Stimulation rate 1/s |
| 15        | 5.67                  | 2.56 | 1.03 | 0.26          | 7.11 |
| 16        | 7.30                  | 5.22 | 3.23 | 2.35 | 1.01 |
| 17        | 5.66                  | 5.78 | 4.06 | 1.85 | 1.33 |
| 18        | 2.55                  | 2.03 | 1.85 | 1.68 | 0.91 | 0.26 |
| 19        | 3.47                  | 2.14 | 2.87 | 2.06 | 1.03 |
| 20        | 2.00                  | 2.61 | 3.02 | 2.47 | 1.50 | 0.91 | 0.41 | 2.63 |
| Mean      | 4.44                  | 3.39 | 2.68 | 1.78 |

* Evaluated from \( (m_1^* - m_2) \).
modifications in quantum size during the EPSP leveling out were ascertained. The results of six experiments carried out on thiamine-deprived preparations are reported in Table III. The actual number of quanta delivered to a single neuron after activation at 1/s of a preganglionic fiber is 4.44 in these experiments, which is not statistically different from 3.96 measured in normal ganglia, but is smaller than the 7.08 calculated in the HC-3-treated preparations. The high quantum content values observed in the presence of HC-3 are presumably related to the postsynaptic curare-like effect of the drug, which results in a decrease in quantum size (10). In these experiments, in fact, some preganglionic fibers could have been considered despite their strong synaptic action (Table II, cells no. 11 and 12), which in normal saline would invariably drive the impaled neuron to its firing threshold.

Measurement of the Presynaptic ACh Store

In the HC-3 experiments in which transmitter synthesis is blocked, the amount of ACh initially present in a nerve terminal can be determined as the sum of the amounts of ACh released to elicit all evoked and miniature EPSP's recorded after the application of the drug. Thus, by extrapolation to the abscissa of the curves representing the time-course of quantum content as a function of stimulation time (Fig. 5), and by taking into account the number of quanta released as spontaneous potentials, it is possible to estimate the presynaptic transmitter store in a preganglionic fiber. This value proved to correspond to 4,000–14,000 transmitter quanta, as indicated in Table IV, and

| TABLE IV |
| --- |
| PRESYNAPTIC TRANSMITTER STORE IN NINE PREGANGLIONIC FIBERS |

| Cell no. | Stimulation frequency* | Presynaptic store‡ |
| --- | --- | --- |
| 6 | 10 | 13,739 |
| 7 | 10 | 8,869 |
| 8 | 10 | 6,270 |
| 9 | 10 | 5,199 |
| 10 | 10 | 7,929 |
| 11 | 10 | 11,769 |
| 12 | 10 | 5,087 |
| 13 | 5 | 11,920 |
| 14 | 5 | 3,648 |

Mean (±SD) 8,270±3,551

* Stimulation rate used to deplete the ACh store.
† Calculated as the total amount of transmitter quanta that can be released on a single neuron by prolonged stimulation after blockade in ACh synthesis by HC-3.
reflects the amount of quanta initially present in only those terminals on a neuron that are activated by stimulation of a single fiber. Two sources of error may affect this estimate of the store size: first, the likelihood that significant amounts of ACh may be lost between the application of HC-3 and the starting of stimulation and recording and, secondly, residual synthesis of ACh may occur to a small degree despite the presence of the drug. As pointed out by Elmqvist and Quastel (10), however, these two sources of error affect the estimate in opposite directions and can be considered to offset each other. The presynaptic store was estimated in the thiamine-depleted ganglia in the same way and on the same assumptions as in the HC-3 experiments, and was found to be $5,410 \pm 2,078$ transmitter quanta (mean of six determinations ± SD).

**DISCUSSION**

Electrophysiological techniques have recently been employed in an attempt to characterize transmitter release at a ganglionic synapse during HC-3 treatment. Bennett and McLachlan (1) studied ACh release from nerve terminals of guinea pig superior cervical ganglia during massive stimulation of the cervical sympathetic trunk while recording the amplitude of the evoked EPSP's and they showed an exponential decline of the mean EPSP amplitude until the EPSP's leveled out completely. These authors provided however no direct evidence that changes in postsynaptic chemosensitivity during HC-3 treatment did not contribute to the decline in synaptic responses and gave no information about the basic mechanism involved in transmitter release. When the nerve terminal is progressively depleted of its transmitter store, the release process can in fact be affected in two different ways resulting in (a) the release by a nerve impulse of a constant number of transmitter quanta of progressively smaller size, or (b) the release of a lower number of quanta of unchanged size. There is evidence that mechanism (a) works at the neuromuscular junction, when stimulation of the motor fiber is carried out in the presence of HC-3 (10, 11, 16). In the present study we interfered with the mechanism of ACh synthesis by impairing the supply of either the acetyl group (thiamine deprivation) or the choline group (exposure to HC-3), both treatments leading to a progressive decline in ACh output from the ganglion associated with a depletion of transmitter tissue content. The results obtained here strongly support the contention that EPSP rundown observed in rat sympathetic neurons during repetitive preganglionic stimulation is solely related to the decrease in the number of transmitter quanta released per impulse and not to any modification in the size of individual quanta. The difference between the results obtained at the neuromuscular junction (10) and ours is very surprising, since one might expect the basic events associated with the release process to be almost similar despite species
and preparation differences. The observations reported here are in line with the original formulation of the quantum hypothesis (17), which states that transmitter quanta can be released by presynaptic terminals only when of full size, and this has been widely confirmed to occur during drastic changes in transmitter release, such as those taking place during the physiological processes of facilitation and inhibition.

The basic assumption of the quantum hypothesis is that the transmitter is stored in the presynaptic terminal in the form of a number \( n \) of discrete packets each having a certain probability \( P \) of release in response to a nerve impulse. One could raise the question as to whether the release process might deviate from the Poisson hypothesis when the terminal is depleted of its transmitter store and presumably the number of preformed quanta is drastically reduced. Johnson and Wernig (15), however, have recently studied the transmitter release mechanism at the neuromuscular junction of the crayfish and showed that with quantum content values comparable to those reported here, if \( P \) is sufficiently low (<0.1), the Poisson model will still provide a good approximation of the release process and \( n \) can be as small as 2.8. The fact that the quantal release is well described by Poisson statistics under the circumstances of reduced synaptic efficacy presented in this paper, provides indirect evidence that \( P \) remains low at the ganglionic synapse despite the fall in the number of available quanta.

There is no evidence that choline acetyltransferase activity is reduced during thiamine deprivation. The amount of enzyme contained in each ganglion was in fact found to be still adequate to synthesize in a test tube 1.5 times the total normal ganglion content of ACh within 1 min (H. Ladinisky et al., in preparation). Furthermore, the supply of choline from the bathing solution is sufficient to support any conceivable rate of ACh synthesis. It appears reasonable therefore to attribute the precise biochemical defect responsible for the impairment observed in synaptic transmission of thiamine-depleted ganglia to reduced synthesis of new acetyl-CoA groups from pyruvate. In unstimulated ganglia the rate of ACh synthesis is normally much faster than the rate of release, the excess transmitter being exposed to hydrolysis by the acetylcholinesterase present within the nerve terminal (2). It is therefore likely that a decrease in the rate of ACh synthesis may occur, at rest or during low frequency stimulation, without altering the levels of transmitter release. The quantum content values were in fact found to be unaffected at 1/s in the thiamine-depleted preparations. In the course of repetitive high-frequency stimulation in cat superior cervical ganglia the rate of ACh release increases by a factor of 70 and that of synthesis by a factor of 7 (2). Under such conditions a relatively small decline in the rate of transmitter synthesis will result in progressive depletion of the ACh content in the tissue, synthesis being inadequate to keep pace with accelerated release. Birks and Mac-
Intosh (2) provided evidence that in the course of diminishing store size, transmitter release is proportional to the amount of ACh in the ganglion. The steep decline in quantum content values observed during prolonged stimulation in thiamine-deprived ganglia is therefore the functional counterpart of the progressive depletion of the ganglion transmitter stores. Thus, the synaptic impairment associated with thiamine deficiency primarily reflects changes occurring in the presynaptic terminals, while the constancy in quantum size makes it extremely improbable that postsynaptic factors, such as receptor desensitization, could be involved in depression of synaptic responses.

The total normal ACh content of rat superior cervical ganglion is 28–30 ng, which is reduced by 85% under prolonged preganglionic stimulation performed in vitro in the presence of HC-3 6 \times 10^{-6} M (H. Ladinsky et al., in preparation). These figures may be perfectly compared with those obtained by Birks and MacIntosh (2) with cat superior cervical ganglia and by Potter (32) in isolated rat diaphragm. Assuming that all the ACh that disappeared from the ganglion came from the nerve terminals and was released on the postsynaptic element, it is possible to evaluate the average amount of transmitter releasable upon a single neuron. This estimate is 2.18 \times 10^4 molecules of ACh, the mean number of cells in the rat superior cervical ganglion being 37,400 (18, 19). From this and the number of molecules of ACh per quantum (tentatively 4.5 \times 10^4 molecules [12]) it would appear that 4.8 \times 10^4 quanta are present in the nerve endings impinging upon a single neuron. One may be tempted to divide this figure by the calculated presynaptic transmitter store in order to obtain a quantitative evaluation of the whole preganglionic input per cell, which corresponds to six afferent fibers. Assuming that the simultaneous release of some 10 quanta is necessary to drive the ganglion neuron to its firing threshold (as derived from a mean unit potential of 1.8 mV), and taking into account the mean quantum content values per impulse reported above, the safety factor for synaptic transmission would appear to be about 4, when the whole presynaptic input is activated.

These attempts to characterize the nervous connections and the synaptic efficacy in rat autonomic ganglia are obviously based on the assumption that the parameters taken into account are not seriously affected by extrapolation from a limited number of observations and that the units explored may really represent an homogeneous sample of the actual ganglion organization.

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