Quantal release at a neuronal nicotinic synapse from rat adrenal gland

(chromaffin cell/synaptic current/acetylcholine receptor/patch-clamp/slice)

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ABSTRACT The neuronal nicotinic synapse in tissue slices of the adrenal medulla was studied with whole-cell patch-clamp. Excitatory postsynaptic currents (EPSCs) were evoked by local field stimulation or occurred spontaneously especially when external $[K^+]$ was increased. EPSCs were carried by channels sharing biophysical and pharmacological properties of neuronal-type nicotinic receptors (nAChRs). A single-channel conductance (γ) of 43–45 pS was found from nonstationary variance analysis of EPSCs. Spontaneous EPSCs were tetrodotoxin-insensitive and Ca^{2+} -dependent and occurred in burst-like clusters. Quantal analysis of spontaneous EPSCs gave a quantal size of 20 pA and amplitude histograms were well described by binomial models with low values of quantal content, consistent with a small number of spontaneously active release sites. However, rare large amplitude EPSCs suggest that the total number of sites is higher and that extrajunctional receptors are involved. Our estimates of quantal content and size at the chromaffin cell neuronal nicotinic synapse may be useful in characterizing central neuronal-type nicotinic receptor-mediated cholinergic synaptic transmission.

The neuronal nicotinic receptor (nAChR) family displays a large diversity of α and β subunit composition and, correspondingly, differences in pharmacological profile, single-channel conductance, and distribution in the central nervous system and autonomic ganglia (1, 2). Activation of central somatic nAChRs either depolarizes neurons from prefrontal cortex (3), medial habenula (4), and locus coeruleus (5) or hyperpolarizes neurons from dorsolateral septal nucleus (6). As well, nAChRs appear to be involved in presynaptic control of transmitter release (7, 8). However, apart from the early discovery of nicotinic excitatory synaptic potentials in cat Renshaw cells (9), postsynaptic nAChRs have not been shown to be directly involved in central synaptic transmission. Thus, neuronal nicotinic synapses have been mostly studied in vertebrate autonomic ganglia (10–13) and recently in the guinea-pig adrenal gland (14, 15). Acetylcholine (ACh) release has been shown to be quantal at ganglionic synapses (16), but detailed quantal analysis was not done due to the poor resolution associated with intracellular recording. Detection of the postsynaptic action of a single quantum is a prerequisite to quantal analysis and determination of quantal parameters (17), thereby allowing study of pre- and postsynaptic factors involved in synaptic transmission (18, 19).

Cholinergic synapses arising from splanchnic nerve terminals are present on chromaffin cells (20). These cells are small, rounded, electrically compact and well suited for patch-clamp studies. ACh-induced current in chromaffin cells is due to the activation of $\alpha 3$ subunit containing nAChRs (21, 22), which are also expressed in the hippocampus, medial habenula, lateral

geniculate, and granular layer of cerebellum (2, 23). The $\beta 2$ and perhaps also the $\beta 4$ subunit appear to be coexpressed in chromaffin cells, given the sensitivity to κ - but not to α -bungarotoxin (24). Our experiments were designed to characterize nAChR-mediated excitatory postsynaptic currents (EPSCs) on chromaffin cells in thin tissue slices from rat adrenal gland. Quantal analysis was also made on spontaneous EPSCs. Our data represent a clear example of quantal analysis applied to a synapse of small quantal size. They may help to identify excitatory cholinergic synaptic transmission in brain regions where ACh-containing fibers and neurons expressing nAChRs are known to occur concomitantly.

METHODS

Adrenal glands were collected from 12-week-old male Wistar rats killed by cervical dislocation. They were placed in cold ($\leq 2^\circ\text{C}$) external solution. After overlying fatty tissue and the capsule were removed, slices ($\approx 300\ \mu\text{m}$ thick) were cut with a vibroslicer (Campden, Sileby, U.K.). Slices were then maintained at room temperature in external solution (140 mM NaCl/2 mM KCl/3 mM CaCl_2 /2 mM MgCl_2 /26 mM NaHCO_3 /10 mM glucose, pH 7.4) and gassed with 95% O_2 /5% CO_2 .

Chromaffin cells were visually identified using an upright microscope and a $\times 40$ water-immersion objective (Nikon). The recording chamber was continuously perfused (1–2 ml/min) with external solution at room temperature (22 – 25°C). Thick-walled borosilicate glass pipettes (1–3 M Ω) were filled with internal solution (140 mM KCl/2 mM MgCl_2 /1 mM CaCl_2 /11 mM EGTA/20 mM Hepes/5 mM MgATP/0.3 mM MgGTP/10 mM phosphocreatine/50 units of phosphohexokinase per ml, pH 7.4 with KOH). Whole-cell recordings (25) with a -60 mV holding potential and seal resistances ≥ 10 – 20 G Ω were made using an Axopatch 200A (Axon Instruments, Burlingame, CA). As for isolated chromaffin cells (25), cell input resistances were 5–10 G Ω ; consistent with the absence of electrical coupling between cells. Uncompensated mean series resistance (R_s) and cell capacitance were 8.7 ± 0.8 M Ω and 8.6 ± 0.3 pF ($n = 30$), respectively. Data were discarded when R_s varied by $> 30\%$. EPSCs were usually filtered (4-pole Bessel) at 1 kHz (-3 dB) and acquired at 3–10 kHz using pClamp (Axon Instruments). Afferent fiber nerve terminals were locally field stimulated with 20 μs voltage pulses (1–50 V; 0.01 Hz) from an isolated stimulator using two 1-M Ω tungsten microelectrodes glued together (tip separation, $\approx 50\ \mu\text{m}$; see

Abbreviations: EPSC, excitatory postsynaptic current; nAChR, neuronal nicotinic receptor; γ , single-channel conductance; ACh, acetylcholine; p , release probability; n , number of release sites; q , quantal size; R_e , survivor function; R_s , series resistance; $B(X = k)$, binomial law; TTX, tetrodotoxin; p.d.f., probability density function; σ^2 , EPSC amplitude variance; σ_q^2 , quantal variance; σ_n^2 , noise variance; τ , decay time constant; ν , synaptic frequency; I – V , current–voltage.

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Fig. 1A). External solutions containing elevated [KCl] (usually 20 mM, substituted for NaCl) or nicotinic antagonists were bath-perfused.

Spontaneous EPSCs were detected and amplitudes were measured by using software provided by P. Vincent (Ecole Normale Supérieure, Neurobiologie, Paris). Only single events were visually selected for construction of amplitude histograms. Nonstationary variance analysis (26) was made on ensembles of $n = 30$ –80 EPSCs of similar amplitude (decay kinetics were not used to select EPSCs as these were homogeneous, and the rising phase of EPSCs was omitted from the analysis). Variance was calculated as $S_{n-1} = \sum_{k=1}^n (i_k + i_{kp} I_{mn})^2 / (n-1)$, where i_k is the k th EPSC function, i_{kp} the peak EPSC value, and I_{mn} the mean EPSC function normalized to -1 . The variance-current relation S_{n-1}/I_{mn} was fitted to $S_{n-1} = i I_{mn} - I_{mn}^2/N$, where I_{mn} is the mean EPSC function, i the unitary current amplitude, and N an integral number of channels. An EPSC reversal potential of -8.2 mV was used in calculating γ .

Stochastic properties of release were examined following Bornstein (27). Series of $n \geq 500$ consecutively occurring EPSCs were detected and the intervals t_k ($1 < k < n$) between EPSCs sorted. The $\ln(\text{Re})$ function was plotted as $\ln(\text{Re}) = \ln[1 - k/(n+1)]$ against t_k (where Re is the survivor function). The plot can easily be compared with a stationary random Poisson process where $\ln(\text{Re}) = -\nu t_k$ with ν being the mean synaptic frequency.

EPSC amplitude histograms were fitted by sums of N Gaussians with no constraints and $3 \cdot N$ free parameters (standard deviation σ , mean m and area for each Gaussian, $1 < N < 6$). This fit was used to estimate starting m and σ values. Convolution fits (18) were then made in two ways by using $\sum_{k=1}^N B(X=k)g_k$, where $B(X=k)$ is a binomial law (with n release sites and p the release probability), with g_k Gaussians associated with N fitted peaks and $3 \cdot N + 2$ free parameters. Real observed EPSC frequencies were used in fitting method 1. As observed frequencies were dependent on large events that had a large sampling error (due to their very low probability), in fitting method 2, the area under all data bins was a free parameter. The fit was considered satisfactory when the data probability density function (p.d.f.) area differed $<0.3\%$ from the convolution fit area [areas were <1 since large events and $B(X=0)$, $B(X>N)$ values were excluded from fits]. This allowed the sensitivity of the fit to n to be tested. Fitted distributions were compared using χ^2 -values and Kolmogorov–Smirnov tests applied to cumulative histograms of unbinned data. Nonlinear least-square fitting procedures were based on the Marquardt–Levenberg algorithm. Student's unpaired t test was considered nonsignificant if $P > 0.1$. Data are given as mean $\pm$ SEM for n cells.

RESULTS

Evoked EPSCs. Recordings were made from visually identified chromaffin cells in the medulla region of thin slices of adrenal gland (Fig. 1A). Under voltage-clamp, inward Na^+ and Ca^{2+} currents and a large outward Ca^{2+} -dependent K^+ current with a N -shaped I - V relation were typically seen (not shown). The mean resting potential under current-clamp was -60.6 ± 2.1 mV ($n = 5$). As nerve fibers invade the medulla in a centripetal fashion with no well-defined fiber tracts in the gland (20), nerve terminals on chromaffin cells were directly field-stimulated using bipolar tungsten electrodes (Fig. 1A). Inward synaptic currents were elicited in an all-or-none fashion as the stimulus intensity was increased (Fig. 1B), with a latency of 4.0 ± 0.5 ms ($n = 5$) and no inflexions in the rising phase (10–90% rise time was 1.7 ± 0.2 ms, $n = 8$), suggesting that a single fiber was stimulated. In a given cell, evoked EPSC amplitude showed little variation (mean/SEM was $<10\%$; see Fig. 5F) and was on average -733 ± 239 pA ($n = 7$). EPSC decay kinetics were well-fitted by a single exponential (Fig. 1C)

FIG. 1. (A) Schematic drawing of a slice of adrenal gland (c, cortex; m, medulla) and the position of the stimulating and recording electrodes. (B) EPSCs were evoked in an all-or-none fashion. (C) EPSCs decayed as single exponentials; $\tau = 10.3$, 9.2, and 4.8 ms at -60 , -40 , and -10 mV, respectively. (D) EPSC peak current-voltage (I - V) relation.

with a voltage-dependent time constant (τ) of 10.6 ± 0.6 ms at -60 mV ($n = 10$). The EPSC peak I - V relation (Fig. 1D) had strong inward rectification and a -8.2 ± 2.2 mV ($n = 3$) reversal potential. EPSCs were reversibly abolished by the nAChR antagonist hexamethonium (Fig. 2B). Nonstationary variance analysis gave $\gamma = 43.1 \pm 2.8$ pS ($n = 3$; Fig. 2C) for

FIG. 2. Properties of evoked (Left) and spontaneous (Right) EPSCs. (A) EPSC decays were single-exponential; the decay time constant τ is indicated. (B) Reversible block by $5 \mu\text{M}$ hexamethonium. (C) Examples of nonstationary variance analysis of 60 evoked and 30 spontaneous EPSCs. N is the number of open channels for an EPSC of mean amplitude, and γ is the single-channel conductance.

the channels carrying evoked EPSCs, close to the $\gamma = 44$ pS for single nAChR currents in chromaffin cells (25). These data confirm that the EPSCs are mediated by nAChRs.

Spontaneous EPSCs. Quantal analysis was not possible on evoked EPSCs due to the very small variations in amplitude with our stimulation protocol. Instead, we focused on spontaneously occurring EPSCs. Because in normal solution these had a low frequency ($\nu < 0.01$ Hz; but in 5 of 100 cells $\nu = 2.7 \pm 2.1$ Hz), external $[K^+]$ was raised to 20 mM. Spontaneous and evoked EPSCs shared similar properties: the spontaneous EPSC decay τ was 13.2 ± 1.4 ms ($n = 12$, -60 mV; Fig. 2A) and both were reversibly inhibited by 5 μ M hexamethonium (Fig. 2B). Complete reversible block of spontaneous EPSCs was also obtained with 60 μ M mecamylamine, 20 μ M β -erythroidine and 100 μ M hexamethonium ($n = 3$ for each drug; data not shown). Nonstationary variance analysis gave a mean $\gamma = 45.0 \pm 5.0$ pS ($n = 6$; Fig. 2C) for spontaneous EPSCs, which was not different from evoked EPSCs.

Non-Independence of Synaptic Events. EPSC activity usually occurred in bursts (Fig. 3A) both in the absence ($n = 3$) and presence of high external $[K^+]$ ($n = 5$). This clustering of events is shown (Fig. 3A) by the non-linearity of the $\ln(\text{Re})$ – EPSC interval plot (27), and the cumulative EPSC interval distribution was different from a stationary random Poisson process (Kolmogorov–Smirnov test, $P < 0.05$). EPSC frequency (ν) was highly dependent on external $[K^+]$ (not shown), likely reflecting presynaptic Ca^{2+} entry. If ν is an indirect indicator of presynaptic $[Ca^{2+}]$, then the clustering of events may be attributable to nerve terminal Ca^{2+} oscillations for three reasons: (i) when $\nu > 10$ Hz, perhaps reflecting a high sustained presynaptic Ca^{2+} level, clustering disappeared and the interval distribution was not different from a Poisson

FIG. 3. Non-independence of EPSC occurrence. (A) EPSCs appear in bursts (mean frequency, ν , is given) and clustering is seen by the deviation of the $\ln(\text{Re})$ curve from a random Poisson process (straight line with slope $\nu = 1.2$ Hz). Re was calculated as $1 - k/(n + 1)$, where k is the rank of the sorted EPSC intervals and n is the total number of EPSC intervals. (B) When $\nu > 10$ Hz, EPSC intervals follow a Poisson process. (C) A transient increase in EPSC frequency occurs after large spontaneous or evoked events. (Inset) At a higher gain, unquantal events ≈ 20 pA are seen in the peak of the evoked EPSC (encircled, left).

process ($n = 3$, $P > 0.2$; Fig. 3B); (ii) when release probability was low, the occurrence of a large (≥ 300 pA) EPSC was followed by an increase in spontaneous EPSC ν , perhaps indicative of a transient rise in presynaptic Ca^{2+} (Fig. 3A and C); and (iii) blocking of presynaptic Ca^{2+} entry by 50 μ M Cd^{2+} reduced ν and EPSC clustering, with the $\ln(\text{Re})$ plot being closer to a Poisson process (Fig. 4E). Thus, EPSCs seem to be independent under both high and low stationary presynaptic Ca^{2+} levels.

Synchronous Release. Since neuronal somata are present in the adrenal medulla (20), the triggering and clustering of spontaneous EPSCs may be dependent on Na^+ action potentials. In 3 of 3 cells, 10 μ M TTX had no effect on EPSC ν , interval distributions, and amplitude histograms (Fig. 4A–C). We conclude that fibers making synaptic contacts with chromaffin cells are preganglionic in origin and that spontaneous synaptic activity is not modified by block of Na^+ current. As Ca^{2+} influx affects EPSC clustering (Fig. 4E), it may also be important in triggering synchronous release of several quanta. Indeed, 50 μ M Cd^{2+} reduced mean EPSC amplitude and ν by 57% and 64% respectively ($n = 3$; Fig. 4D–F), with release being completely and reversibly inhibited at 200 μ M ($n = 4$; data not shown). In 50 μ M Cd^{2+} , amplitude histograms were no longer skewed (Fig. 4F) and were well described by a single Gaussian with a mean ≈ 20 pA (data not shown). These EPSCs likely reflect unquantal release, indicating a quantal size $q \approx 20$ pA at this synapse. EPSCs > 20 pA in amplitude likely result from synchronous release of several quanta triggered by a rise in presynaptic Ca^{2+} . In agreement, EPSC rise time and am-

FIG. 4. (A–C) Tetrodotoxin (TTX; 10 μ M) did not affect mean EPSC amplitude, frequency, and clustering. The $\ln(\text{Re})$ slope and amplitude histogram (p.d.f.) were unchanged after TTX. (D–F) Cd^{2+} (50 μ M) decreased mean EPSC amplitude, frequency and clustering. After Cd^{2+} , $\ln(\text{Re})$ is closer to a random Poisson process with a smaller slope, and large amplitude EPSCs are absent. (G) Lowering external $[Ca^{2+}]$ alters synchronization of evoked release. $[Mg^{2+}]$ was raised to 4.8 mM when $[Ca^{2+}]$ was 0.2 mM.

plitude were not correlated (data not shown). Also, when external Ca^{2+} was lowered to 0.2 mM, the variability of the latency and rise time of evoked EPSCs was increased (Fig. 4G), which is consistent with a role for Ca^{2+} entry in synchronizing release.

Estimation of Quantal Parameters. Spontaneous release was further subject to quantal analysis. Quantal size (q) was estimated for cells having unimodal EPSC distributions from the mean (19.6 ± 0.9 pA; $n = 3$) of fitted Gaussians. In such cases, the noise variance (σ_n^2) was not different from the peak EPSC amplitude variance (σ^2 ; F -test) and thus, quantal variance ($\sigma_q^2 = \sigma^2 - \sigma_n^2$) was negligible. For some cells (Fig. 5B–D and Table 1), amplitude histograms showed clear peaks, presumably due to multiquantal release. The mean peak separation was then determined from fits with sums of Gaussians giving another estimate of $q = 18.6 \pm 0.7$ pA ($n = 11$; Fig. 5C and D, Left).

The number of active release sites (n) and the mean release probability (p) were estimated in nine cells where ν and mean EPSC amplitude were stable. In all cells, data were well described by a convolution fit (18) based on a binomial model (Fig. 5C and D, Right). The mean peak separation was 19.13 ± 0.45 ($n = 9$, Table 1), close to the value from fits with multi-Gaussians (Fig. 5C and D, Left). However, such bino-

mial convolution fits were sensitive to initial n values, and for this reason, two different methods were used. For cell 3 of Table 1 (Fig. 5C, Right), both methods gave fits with minimal χ^2 values for $n = 4$ (Fig. 5E). For cell 8 of Table 1 (Fig. 5D, Right), data were best fitted with $n = 8$ using method 1 and $n = 7$ with method 2 (Fig. 5E). This indicates that the number of spontaneously active sites is low, as seen both in the presence and absence of high $[\text{K}^+]$ (Table 1).

Large amplitude EPSCs (≥ 300 pA, the largest being >1 nA) having similar rise times compared with unquantal EPSCs (≈ 20 pA) indicate that the total number of release sites may well be higher: $n \geq 50$ (even if $p \approx 1$ is assumed). As well, using a binomial model, the variance of evoked EPSCs is best fitted with $n > 20$ (Fig. 5F). Thus stimulation producing large EPSCs may recruit sites that are not spontaneously active. However, if q is determined by postsynaptic channel density, then activation of extrajunctional nAChRs may also contribute to large EPSCs. This may explain the biexponential EPSCs > 800 pA with a slow component ($\tau > 50$ ms) seen here, as proposed by Rang (10). We conclude that even when external $[\text{K}^+]$ is raised, only a fraction of release sites is spontaneously active, with others having $p \ll 1$. This view is supported by low fitted values of quantal content ($n \cdot p$; Table 1) even when $\nu > 10$ Hz in high $[\text{K}^+]$. The total number of release sites is at least 5-fold greater than the average number of 3–4 synaptic boutons seen on individual rat chromaffin cells (20).

DISCUSSION

Whole-cell patch-clamp recordings from chromaffin cells in thin slices of rat adrenal gland have allowed us to characterize EPSCs and synaptic transmission mediated by neuronal nicotinic receptors. The sensitivity of EPSCs to hexamethonium, mecamylamine, β -erythroidine, the EPSC reversal potential of approximately -10 mV, the strong inward rectification and the ≈ 45 pS single-channel conductance are all characteristic of nAChRs. The close match between EPSC decay τ and mean open burst time of chromaffin cell nAChRs (28) indicates that on average, ACh binds once during an EPSC, with the EPSC decay phase being determined by single-channel closure kinetics, and that desensitization is negligible (14, 24, 28).

EPSCs were clearly TTX-insensitive and Ca^{2+} -dependent, as Cd^{2+} reversibly abolished release. The most accurate estimate of q (19.6 pA) was from unimodal EPSC distributions where σ_q^2 was negligible compared with σ_n^2 , as observed for dorsal lateral geniculate nucleus (29) and hippocampal pyramidal neurons (30). Only unquantal events were detected in these cases. Amplitude histograms of γ -aminobutyric acid- and glutamate-mediated miniature synaptic currents were always skewed in hippocampal pyramidal cells (31–33), probably due to electrotonic filtering. In contrast, here both EPSC decay τ and rise time were homogeneous, likely reflecting the simple geometry of chromaffin cells. Amplitude histograms in some cells had distinct peaks reflecting multiquantal events. This was seen rarely in granule cells (34) and more frequently in dorsal lateral geniculate nucleus neurons which are electrically compact and receive synaptic inputs from homogeneous electrotonic distances (29). The low variability in q (Table 1) is striking compared with other studies (29–34) and strongly suggests that q is homogeneous for different sites. The small value of q (≈ 20 pA) and its small variability suggest that q is determined by a low density of nAChRs.

Release of 1 quantum of ACh will open 7–18 nAChRs, and given the high input resistance of chromaffin cells, a spike should always be produced (indeed, opening of 1 channel by ACh is known to be sufficient; ref. 25). While spontaneous synaptic activity was generally absent, EPSCs when present should trigger both action potentials and catecholamine release (35). The mean size of evoked EPSCs indicates that this synapse has a large safety factor, as found in guinea-pig

FIG. 5. Quantal analysis of spontaneous EPSCs. (A) Unimodal amplitude histogram of EPSCs in high $[\text{K}^+]$ with mean amplitude (q) from a fitted Gaussian p.d.f. (B) Selected multiquantal EPSCs (Upper) and averages (Lower) from the cell in D. (C) Amplitude histogram for a cell ($R_s = 3$ M Ω , $\nu = 1.3$ Hz) with four peaks. Superimposed fit for four summed Gaussians (Left) and a binomial convolution fit (Right). (D) Amplitude histogram for a cell ($R_s = 6.5$ M Ω , $\nu = 0.3$ Hz) with six peaks. Superimposed fit for six summed Gaussians (Left) and a binomial convolution fit (Right; EPSCs > 108 pA were omitted to make the convolution fit). (E) χ^2 -values for binomial convolution fits for different numbers of release sites (n) for cells in C (▼, ●) and in D (▽, ○) for method 1 (▽, ▼) and method 2 (○, ●). (F) Amplitude histogram of evoked EPSCs with three superimposed binomial convolution fits: $n = 20$, $p = 0.6$ for 1; $n = 50$, $p = 0.24$ for 2; $n = 180$, $p = 0.07$ for 3; $q = 20$ pA and $n \cdot p \cdot q = 240$ pA for 1–3.

Table 1. Quantal parameters for spontaneously occurring EPSCs

Cell	No. of peaks	q , pA	n	p	$n \cdot p$	$n \cdot p \cdot q$, pA	ν , Hz	No. of EPSCs
1	2	21.39	—	—	<0.05	—	1.83	582
2	2	18.22	—	—	0.09	—	0.55	521
3*	4	19.46 $\pm$ 0.80	4	0.45	1.81	35.22	1.30	525
4	4	19.65 $\pm$ 1.65	4	0.57	2.28	44.80	30.09	768
5	4	19.19 $\pm$ 1.44	4	0.60	2.40	46.06	1.05	324
6	4	16.38 $\pm$ 0.67	4	0.63	2.50	41.02	20.69	656
7	5	19.93 $\pm$ 0.85	6	0.41	2.46	49.03	2.87	1016
8*	5	18.57 $\pm$ 0.18	7	0.44	3.07	57.06	0.30	180
9	6	19.40 $\pm$ 0.18	6	0.43	2.58	50.05	2.97	1821
		19.13 $\pm$ 0.45			0.50 $\pm$ 0.03			

Quantal size q , number of active release sites n , and mean release probability p were derived from binomial convolution fits (18). For cells 1 and 2, $n \cdot p$ was estimated from $B(X = 1)/B(X = 2)$. $B(X = k)$ is a binomial law; ν is EPSC frequency.

*Cells in normal external $[K^+]$.

chromaffin cells (14, 15). Thus fast excitation at this synapse is mediated by nAChRs that are spontaneously silent, a general feature of ganglionic nicotinic transmission (10). Upon splanchnic nerve stimulation, large EPSCs will depolarize chromaffin cells for long periods, since $\tau > 50$ ms for EPSCs > 800 pA, allowing strong Ca^{2+} entry through both voltage-dependent Ca^{2+} channels and nAChRs (36) and sustained release of catecholamines (35).

Spontaneous EPSC activity was well described by binomial quantal models with a low number of active release sites. Rare large EPSCs indicate that the total number of sites is higher even if we assume that extrajunctional nAChRs are involved. This suggests that different populations of release sites exist, some with $p = 0.1$ – 0.8 and others with $p \ll 1$, as found in hippocampal neurons in culture and in slices (37, 38). However, the total number of sites is difficult to quantify accurately because EPSC populations were too small. Nevertheless q and $n \cdot p$ were determined precisely and will likely be valuable in characterizing modulation of ACh release at this synapse and perhaps also at other central cholinergic synapses. Interestingly, the values of these parameters are in the range of those found for other central neurotransmitters.

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