LCMV (Fig. 4) (25). Lysis of NK-sensitive yeast artificial chromosome (YAC) targets was reduced by a factor of 3 to 9 in IL-$2^{-/-}$ mice as compared with control mice.

The fact that NK cell responses were impaired but inducible in IL-2–deficient mice confirms the previous finding that IL-2 supports proliferation of NK cells (8) but that interferon-α (IFN-α) and IFN-β may be more important in the induction of the NK cell response (26).

Taken together, in vivo CTL responses against vaccinia virus and LCMV as well as B and T_H cell responses against VSV were normal or only slightly impaired. Not only were antigen-specific CTL and T_H cells readily induced, but in vivo proliferation occurred: In uninfected mice, the frequency of vaccinia- or LCMV-specific CTLs is less than 10^{-6} spleen cells. During infection, this frequency increases as a result of proliferation. For primary ex vivo cytotoxicity to be observed, the frequency of virus-specific CTLs must reach the range of 10^{-2} (20, 27). Thus, the fact that primary in vivo cytotoxicity was generated in IL-2–deficient mice suggests that proliferation on the order of 10,000 has occurred despite the absence of IL-2. The mildness of the effects on in vivo T and B cell responses against the viruses tested was unexpected. The notion that IL-2 plays a central and limiting role in immunoregulation is based mostly on studies in vitro and is confirmed here for secondary CTL responses in vitro. IL-2 receptor γ chain mutations have recently been shown to result in severe combined immunodeficiency in humans (28). In contrast to the IL-$2^{-/-}$ mice, T cells in these patients are diminished in numbers and are apparently unresponsive. These differences suggest either that IL-2 has a more important role in human T cell development and function or that the IL-2 receptor γ chain has additional functions, for example, as a component of other cytokine receptors.

In conclusion, in vivo immune responses in IL-2–deficient mice question the importance of IL-2 as defined by in vitro studies. It appears that, in vivo, other lymphokines may compensate for the IL-2 defect.

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A Ca-Dependent Early Step in the Release of Catecholamines from Adrenal Chromaffin Cells

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Intense stimuli, such as trains of depolarizing pulses or the caffeine-induced release of calcium from intracellular stores, readily depress the secretory response in neuroendocrine cells. Secretory responses are restored by rest periods of minutes in duration. This recovery was accelerated when the concentration of cytosolic calcium was moderately increased and probably resulted from calcium-dependent replenishment of a pool of release-ready granules. Continuously increased concentrations of calcium led to the overfilling of such a pool. Subsequently, secretory responses to stronger calcium stimuli were augmented. Hormone-induced calcium transients with a plateau phase of increased concentration of calcium may enhance the secretory response in this way.

The docking of secretory vesicles at active zones is common to most neuronal synapses (1). It allows for rapid secretion during synaptic transmission. Recent evidence suggests that neuroendocrine cells also have a small pool of granules that can be released more quickly than the rest (2–6). When the intracellular concentration of Ca^{2+} $[Ca^{2+}]_i$ rises, secretion occurs with several distinct kinetic phases (5, 6). We described a model (7) that can simulate different types of secretory experiments under the simple assumption of a two-step process: Granules migrate during maturation from a reserve pool A to a release-ready pool B, the latter being the source of secreted vesicles. To accommodate all the available data, we had to assume that both steps in this scheme are influenced by $[Ca^{2+}]_i$. The model predicted that the size of the release-ready pool should increase with increasing $[Ca^{2+}]_i$ up to about 0.5 μM, resulting in the augmented secretion to subsequent stimuli. Such an augmentation has also been inferred from biochemical measurements (4) and from the amplitudes of kinetic components in experiments with caged calcium (6). Here we investigated the effects of $[Ca^{2+}]_i$ on the release-ready pool of vesicles

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by studying secretory depression (depletion of the pool of release-ready granules) and recovery from depression at different $[Ca^{2+}]_i$ values.

Short trains of depolarizing pulses readily depress secretory responses in adrenal chromaffin cells (Fig. 1A). Five step depolarizations of 200-ms duration were given at intervals of 1 s in each example shown (8). Secretion, which occurred predominantly during the depolarizations, resulted in steplike changes in capacitance. In some cases (such as the example numbered 1 in Fig. 1A), secretion also occurred between pulses, appearing as upwardly sloping segments. Usually, the steplike increases were largest for the first or second pulse of the train and smaller for later pulses. The average capacitance increment was 81 ± 8.3 fF for the first and 27 ± 2.3 fF for the fifth stimulus in a train of depolarizing pulses (mean ± SEM, n = 112). In spite of the general decrease in secretory response, some cells showed a facilitated response (see example 3), as has been shown for pituitary cells (9). The variability in responses may have resulted from differences in the underlying basal $[Ca^{2+}]_i$ and calcium currents (I_{Ca}). Cells with facilitation often had small I_{Ca} but slowly increasing $[Ca^{2+}]_i$ during the train. The average pattern of capacitance changes can be understood on the basis of the model of Heinemann and co-workers (7), in which similar trains of stimuli deplete the pool of release-ready granules to 10 to 50% of its basal size.

The depression of secretory responses can also occur for exocytosis triggered by Ca release from intracellular stores. Although bradykinin or caffeine often induce only small increases in $[Ca^{2+}]_i$, these agents sometimes produce large calcium transients (Fig. 1B). Caffeine application produced a transient larger than 2 μM (10), accompanied by a capacitance increase of about 350 fF. The size of the pool of release-ready granules was tested before and after the caffeine application by means of depolarizing pulses (200 ms). Although these pulses induced similar Ca transients, the secretory response elicited after the caffeine stimulus was much smaller than that elicited before the stimulus. Depression was evident during the caffeine-induced Ca transient in that the rate of secretion dropped well before $[Ca^{2+}]_i$ started to decline. This difference is demonstrated by plotting the rate of capacitance increase against $[Ca^{2+}]_i$ (Fig. 1C). The pronounced hysteresis shows that for the same $[Ca^{2+}]_i$, the rates of exocytosis were higher during the rising than during the falling phase of the response. In nine cases, exocytosis in response to a depolarizing pulse diminished after caffeine treatment from 249 ± 79 fF to 153 ± 64 fF. In all these cases, plots of dC/dt versus $[Ca^{2+}]_i$ displayed hysteresis. The secretory rates for half maximal $[Ca^{2+}]_i$ decayed from 67 ± 22 fF/s to 6.5 ± 2.8 fF/s. For small caffeine-induced transients, after which there was little or no reduction in secretory responses, there was no such hysteresis [the discrepancy between the rising and falling phases was smaller than 15% (five cells)]. Our interpretation of this behavior is that during a large transient the number of release-ready granules diminishes, thus reducing the rates of secretion for a similar calcium stimulus. A similar behavior was described (11) for rhythmic exocytosis in gonadotrophes.

To rule out calcium-dependent inactivation of calcium currents as the basis for secretory depression (12), we designed conditions in which the current increased from pulse to pulse within a train. We applied six depolarizing pulses separated by 1-s intervals, stepping to potentials from −25 to 0 mV in +5 mV increments (13). For the first three pulses the amount of exocytosis (37 ± 9 fF for the first pulse and 132 ± 34 fF for the third pulse, n = 7) increased concomitantly with the magnitude of the calcium current (36 ± 5 pA for the first pulse and 66 ± 5 pA for the third pulse). For later pulses, secretion decreased in amplitude (83 ± 14 fF for the sixth pulse) although the calcium current increased further (79 ± 3 pA for the sixth pulse), thus ruling out calcium current inactivation as a cause for depression.

The model by Heinemann and co-workers (7) predicts that once depression has occurred, the pool of release-ready granules should recover within one to several minutes and that the rate of recovery should be increased at elevated values of $[Ca^{2+}]_i$. This prediction was confirmed experimentally (Fig. 2, A and B).

When 60 s was allowed for recovery after a train of pulses with complete depression, a sizable response similar to the first was again obtained. When $[Ca^{2+}]_i$ was slightly increased by moderate depolarization (to −35 mV), recovery was enhanced. This augmentation occurred despite the fact that often some secretion occurred during the episode of elevated calcium, drawing on the pool of release-ready granules. In 34 paired experiments the size of the total capacitance response was 250 ± 29 fF (mean ± SEM) at basal $[Ca^{2+}]_i$ and 300 ± 31 fF for elevated $[Ca^{2+}]_i$ (Fig. 2B). Augmentation was more pronounced in perforated-patch recordings than in whole-cell measurements (Fig. 2B), which indicates that augmentation was not caused by the loss of factors from the cytosol (14).

Our model (7) predicts that plateaus of elevated calcium, commonly observed after hormone-induced calcium release from intracellular stores, should be particularly effective in the augmentation of the secretory response because they are sufficiently long in duration and of appropriate amplitude. We tested this effectiveness by applying trains of depolarizing pulses before and after exposing the cells to histamine (20 μM) for approximately 60 s (Fig. 2A). Histamine caused the release of calcium, followed by a long-lasting plateau of increased $[Ca^{2+}]_i$. The total capacitance response to depolarizing pulses after the histamine application was larger than that before, although the calcium signal was smaller and appreciable secretion occurred during the histamine application. A third train of pulses after an equal-length recovery period at basal $[Ca^{2+}]_i$ again gave a smaller response than that before this period. In 10 experiments

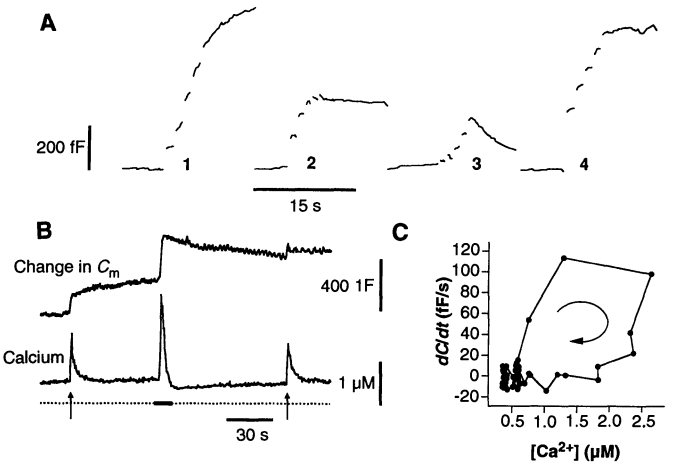

Fig. 1. Depression of secretory responses. (**A**) Trains of five 200-ms depolarizing pulses (from −70 mV to 0 mV), separated by 1-s intervals, were applied. Four examples from different cells display the range of capacitance responses. Capacitance values were sampled at 200-ms intervals. During depolarizing pulses, which occur within one sample interval, the measurement is not reliable. The corresponding data points were eliminated from the record. Between pulses, clusters of four to six sampled values are seen, which indicate that capacitance changes in a steplike fashion during pulses (26). (**B**) Changes in the cytosolic $[Ca^{2+}]_i$ (calcium) and membrane capacitance (change in C_m) in response to either depolarizations (200 ms from −70 to +0 mV; arrows) or applications of 10 mM caffeine (bar), by way of an application pipette placed close to the cell. (**C**) Rate of secretion (dC_m/dt) from a small segment of the traces in (B) around the caffeine-induced transient plotted against the calcium concentration for each data point. The data points display a clockwise hysteresis (arrow indicates time progression).

in which the same protocol was followed, responses after histamine applications were 299 ± 40 fF as compared to 206 ± 23 fF for those without preceding histamine applications. These values together with model predictions for such an experiment are shown in Fig. 2C. When $[Ca^{2+}]_i$ between trains was increased by slight depolarization rather than histamine application, secretory responses displayed a similar Ca^{2+} dependency (Fig. 2C).

To exclude the possibility that augmentation is due to the recruitment of additional calcium current (*15*) or changes in cellular calcium buffer capacity (*16*), we measured calcium current, average $[Ca^{2+}]_i$, and capacitance increases in paired experiments comparing control responses with augmented ones (*17*). Whereas the capacitance signal increased after depolarization-induced augmentation to 190 ± 58% (mean ± SEM, n = 7), calcium current and average calcium signal were reduced to 69 ± 13% and 67 ± 12% of their control values, respectively. Thus, there is no recruitment of extra calcium current. Because the current and the calcium signal dropped by about the same proportion, changes in calcium buffer capacity can be excluded as a cause of the effect as well.

Because the patch-clamp capacitance technique measures overall changes, whether from exocytosis or endocytosis, in the cell surface area, we confirmed the catecholamine release pattern with the carbon fiber technique (*18*, *19*). Figure 3A shows amperometric current measured by a carbon fiber and its integral during a pulse protocol similar to the one applied for capacitance measurements. Between pulse trains the cell was slightly depolarized, which caused some secretion to occur, as evidenced by noise in the current trace. When viewed at higher resolution, single secretory events like those described by Chow *et al.* (*19*) were observed. As in the case of capacitance measurements, depolarization and a concomitant increase in basal $[Ca^{2+}]_i$ caused augmentation, increasing the secretory response from 117 ± 16 pC (n = 11) to 198 ± 18 pC (n = 10), or by 68%. Capacitance responses measured simultaneously were augmented by 70% in this batch of cells.

A more detailed analysis, however, revealed that there are some differences between the two methods of measuring exocytosis. Capacitance records and the integral of the simultaneously measured amperometric current were superimposed (Fig. 3B). The two records were normalized to give the same final amplitude. As in previous cases, five depolarizing pulses were given at 1-s intervals. Although the signals followed the same pattern, there was more depression (relative decrease in successive responses) in the capacitance trace than in

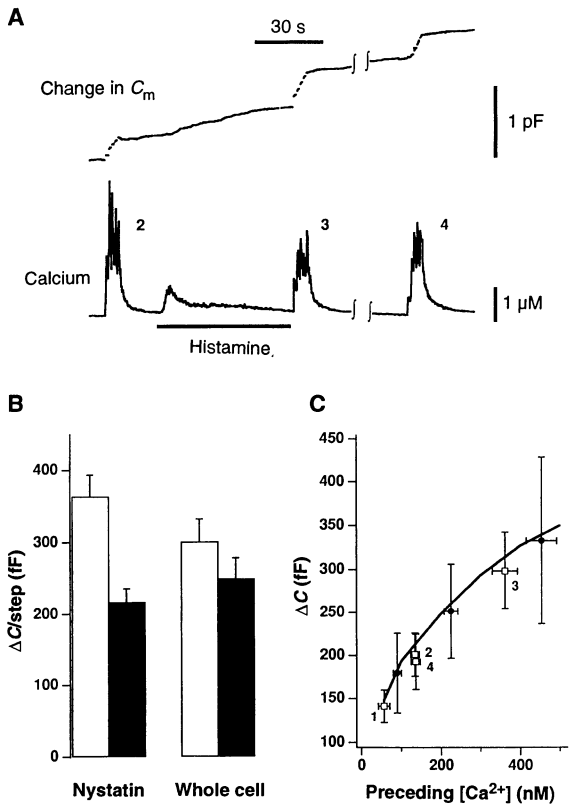

Fig. 2. Augmentation of secretory responses. (**A**) Cytosolic calcium and change in C_m for a time segment in which three trains of five depolarizing pulses each were applied. Trains are marked by numbers 2, 3, and 4 for reference in (C). In the segment between pulse trains 2 and 3, histamine (20 μM) was applied to the cell for 50 to 60 s as indicated by the bar. The interval between trains 3 and 4 (not fully shown) had the same length (approximately 90 s) as the previous one. (**B**) Responses to trains of five pulses each were measured either for control conditions (solid bars) or after slight depolarization (to around −35 mV), thereby enhancing pre-train $[Ca^{2+}]_i$ for 30 s (open bars). Error bars represent the SEM (n = 27). Depolarization alternated with controls (continuous holding potential of −70 mV) during the 60-s intersegment between pulse trains. Depolarizations were discontinued several seconds before the train to allow basal $[Ca^{2+}]_i$ to drop to almost the control values just before the actual train was applied. The experiments were performed either by the perforated-patch technique (Nystatin) or the whole-cell mode (whole cell). (**C**) Data similar to those of (A) from 10 cells were analyzed by averaging the total capacitance increase during trains (open squares). The number 1 marks the average total capacitance increase during the first pulse train in a given cell. The number 3 marks the response to the third pulse train, which was always preceded by a histamine application. Numbers 2 and 4 mark control values for the second and the fourth pulse trains, respectively, which were not preceded by histamine applications. For comparison between histamine- and depolarization-induced augmentation, a restricted set of experiments was analyzed in which timing and degree of depolarization were adjusted so that the resulting calcium changes were similar to the histamine-induced plateaus (filled circles). Error bars represent the SEM of seven to 12 binned data points. The solid line represents model predictions for which a set of parameters was used, which correctly described the average response to 112 pulse trains (*27*).

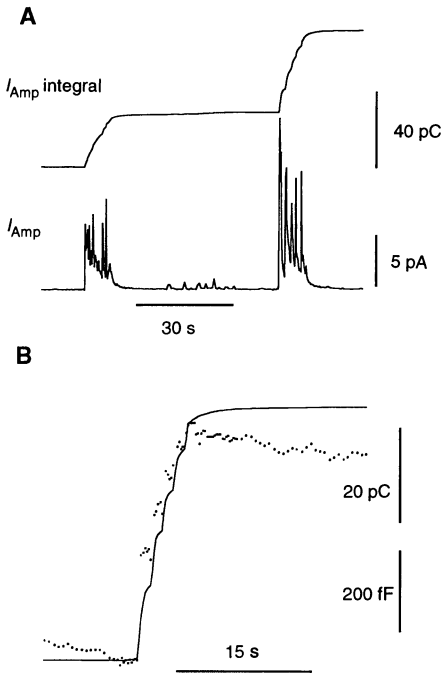

Fig. 3. Comparison between capacitance measurements and the amperometric release assay (*28*). All experiments were performed in the perforated whole-cell mode. (**A**) An example of the amperometric current and its integral for a time spanning two trains of depolarizing pulses. Between the two trains the cell was depolarized for about 30 s, which caused amperometric signals to occur at low frequency. Amperometric responses in pulse trains were much larger after the depolarizing episode (second train) as compared to those in the first train, in which no such preceding depolarization was applied. (**B**) The integral of an amperometric current (solid trace) compared with the simultaneously recorded capacitance increase (dotted trace) during a train of five depolarizing pulses after the enhancement of basal calcium. Differences in relative step sizes between the amperometric current integral and the capacitance signal are apparent. Furthermore, some endocytotic activity can be observed on the capacitance trace both before and after the train.

the amperometric one. Also, after the pulse train a slow decrease in capacitance was observed, whereas the amperometric signal kept increasing for a few seconds. The decrease in capacitance, due to endocytosis, is typical but variable from cell to cell. The measurements in Fig. 3 were performed in the nystatin configuration (8), which consistently shows more endocytosis than that of whole-cell recording (20). Differences between the two traces are also expected because capacitance measurement is applied to the whole cell, whereas amperometry records release with high time resolution from about one-tenth of the surface area, together with some fraction of release from the rest of the cell after variable diffusional delays. These methodological biases explain some of the differences observed between the two methods of measuring depression. However, there may be a real discrepancy, as would occur, for example, if readily releasable vesicles had less hormone content than other ones.

We have described a number of features of secretory responses in terms of changes in the size of a pool of release-ready granules. All of these features qualitatively agree with the two-step model (Fig. 4) that we proposed in (7). In that model, release-ready granules exocytose at a rate proportional to the third power of $[Ca^{2+}]_i$. Vesicles are supplied to the release-ready pool at a calcium-dependent rate that increases in a Michaelis-Menten–like fashion. The particular form of calcium dependencies causes the size of the release-ready pool to increase with $[Ca^{2+}]_i$ up to the point at which the secretion rate surpasses the supply rate. For even higher $[Ca^{2+}]_i$, the pool size drops precipitously as a result of the steeply increasing release rate. The data presented confirm the prediction of augmented release (increased pool size) after moderate increases in $[Ca^{2+}]_i$. We showed this relation both for the case of depolarization-induced increases in $[Ca^{2+}]_i$ and for increases after the histamine-triggered release of intracellular Ca^{2+} stores. The fact that two quite distinct mechanisms of elevating $[Ca^{2+}]$ led to similar augmentation is strong evidence that calcium, rather than depolarization or hormone action (or inositol trisphosphate), causes augmentation. The model predicts that a long-lasting plateau phase of moderately elevated $[Ca^{2+}]_i$ is particularly efficient in overfilling the release-ready pool. Thus, the concept of release control proposed here assigns functional significance to this particular feature of calcium-release transients commonly observed in neurons and neuroendocrine cells after phosphoinositide–mediated responses to hormones or neurotransmitters (21).

Our data confirm and extend the results

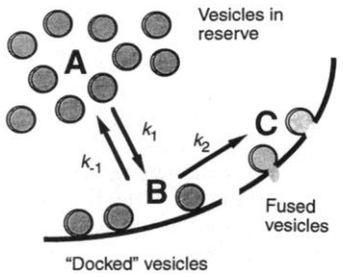

Fig. 4. Scheme of the model proposed in (7). In this model vesicles are secreted with a rate k_2 from pool B (representing release-ready granules) into pool C (secreted granules). Pool B is replenished from a larger pool A (vesicles on reserve). Only about 1% of all the vesicles of a chromaffin cell are in pool B at rest. Rate values:

$$k_1 = \frac{a_1[Ca^{2+}]_i}{b_1 + [Ca^{2+}]_i},\ k_{-1} = a_2,\ k_2 = a_3[Ca^{2+}]_i^3$$

of Bittner and Holz (4), who demonstrated a role of $[Ca^{2+}]_i$ in the priming of secretory granules for release. These investigations and the work of Hay and Martin (22) provide strong support for the conclusions drawn here. Our present data do not allow inferences about the molecular nature of the postulated steps. However, in the light of biochemical data (23–25), the high-affinity calcium-dependent priming process may be tentatively associated with interactions between vesicles and the cytoskeleton, whereas the steeply calcium-dependent second step likely represents the actual membrane fusion process. Nevertheless, our data are equally compatible with two calcium-regulated biochemical processes operating in sequence on already docked granules or with the possibility that the calcium-trigger mechanism undergoes a desensitization-resensitization process.

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13. Holding potential for the experiments was −70 mV, and the pulse duration was 200 ms. Capacitance increases for every depolarizing step were analyzed (Fig. 1), averaged, and compared with the simultaneously measured calcium current during each step. Only a certain fraction of the cells had calcium currents with amplitudes in a suitable range. A major fraction of the cells had currents that saturated the amplifier during the measurement (at a high gain of 50 mV/pA), thus obscuring the determination of actual calcium current values. In a few cells, calcium currents were very small (maximum I_{Ca} < 50 pA), leading to a reversed behavior, namely an increase in capacitance responses during the whole train. This result is expected because small stimuli do not deplete vesicle pools, such that the increase in stimulus strength with increasing depolarization dominates the overall effect. Therefore, analysis was limited to cells with 50 pA < maximum I_{Ca} < 200 pA. Current traces were low-pass–filtered digitally at 100 Hz to remove the sine-wave component. The average current between 25 and 175 ms into the pulse was determined. A similar result of depression in secretory responses despite increasing currents was obtained when the current was increased by stepping down the potential along the positive branch of the U-shaped current-voltage curve of Ca currents (from +25 to 0 mV).
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17. To investigate the effects of changes in the calcium current on the process of augmentation, three

quantities were measured for a given train: (i) calcium currents during the first pulse, (ii) the increase in membrane capacitance during this pulse, and (iii) the average $[Ca^{2+}]$ between the first and second pulses of these trains. These parameters have been compared for trains after augmentation (with slightly enhanced basal calcium between trains) with controls (no previous enhancement of basal calcium). Controls are set to 100%.
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26. To measure step responses (during pulses), linear regressions were performed to three to four data points both before and after a pulse. These fits were extrapolated to the time of the pulse, and their difference was adopted as the step size. The calculation of the derivative of the capacitance trace, as in Fig. 1C, was made by the transferral of short segments of data to the IGOR program (WaveMetrix, Lake Oswego, OR).
27. To simulate responses to pulse trains, the parameters a_1, b_1, and a_3 of the model by Heinemann and co-workers (*7*) have been used unchanged. Peak $[Ca^{2+}]$ at the "release sites" and increments in basal $[Ca^{2+}]_i$ during trains of depolarizing pulses were set to 4.2 μM and 0.42 μM, respectively. A value of 3.45 was adopted as the time constant of $[Ca^{2+}]_i$ decay after pulses, according to experimental results. The constant a_2 for the rate of the depriming of vesicles was increased to 0.012 s^{-1}, and the size of pool A was set to 4000 fF, resulting in a pool B of 230 fF at a $[Ca^{2+}]$ of 100 nM. With these new parameters, we obtained both the correct ratio of capacitance increases between pulses one and five in a train and the experimental result for total secretion during a train of pulses starting from basal $[Ca^{2+}]_i$.
28. All experiments were done in the perforated-patch configuration with the use of nystatin. Amperometric currents were measured as described in (*19*). The voltage at the carbon fiber was continuously held at +800 mV, and the resulting current was measured with an EPC-7 patch-clamp amplifier (List-Electronic, Darmstadt, Germany), filtered at 20 Hz, and sampled by the data acquisition program. Amperometric traces were transferred to the IGOR analysis program for the calculation of current integrals. Before integration, a base line (from a stretch of data without secretory events) was subtracted.
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Inhibition of the EGF-Activated MAP Kinase Signaling Pathway by Adenosine 3′,5′-Monophosphate

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Mitogen-activated protein (MAP) kinases $p42^{mapk}$ and $p44^{mapk}$ are activated in cells stimulated with epidermal growth factor (EGF) and other agents. A principal pathway for MAP kinase (MAPK) activation by EGF consists of sequential activations of the guanine nucleotide exchange factor Sos, the guanosine triphosphate binding protein Ras, and the protein kinases Raf-1, MAPK kinase (MKK), and MAPK. Because adenosine 3′,5′-monophosphate (cAMP) does not activate MAPK and has some opposing physiologic effects, the effect of increasing intracellular concentrations of cAMP with forskolin and 3-isobutyl-1-methylxanthine on the EGF-stimulated MAPK pathway was studied. Increased concentrations of cAMP blocked activation of Raf-1, MKK, and MAPK in Rat1hER fibroblasts, accompanied by a threefold increase in Raf-1 phosphorylation on serine 43 in the regulatory domain. Phosphorylation of Raf-1 in vitro and in vivo reduces the apparent affinity with which it binds to Ras and may contribute to the blockade by cAMP.

Signal transduction pathways used by EGF include activation of $p42^{mapk}$ [or extracellular signal–regulated kinase 2 (ERK2)] and $p44^{mapk}$ (or ERK1), which are closely related protein serine-threonine kinases regulated by dual tyrosine and threonine phosphorylation (*1*). MAPK is likely to mediate at least a subset of cellular responses to EGF, including activation of a number of cytosolic enzymes (phospholipase A_2 and 90-kD ribosomal protein S6 kinase) and nuclear transcription factors (including c-Myc and $p62^{TCF}$) (*1*).

Recent studies have defined several steps in a MAPK activation pathway mediated by Ras. EGF induces autophosphorylation of the EGF receptor (EGFR) on tyrosine residues, creating specific binding sites for Src homology 2 (SH2)–containing proteins, including a multiprotein complex that activates Ras (*2*). The guanine nucleotide–releasing protein Sos, the mammalian homolog of the *Drosophila* protein Son of sevenless, is complexed in the cytoplasm to an SH2-containing adapter protein, growth factor receptor–bound protein 2 (Grb2). After EGFR tyrosine phosphorylation, the Grb2-Sos complex is recruited to the receptor by the SH2 domain of Grb2; localization of Sos with its membrane-bound target Ras catalyzes formation of the active, guanosine triphosphate (GTP)–bound form of Ras. Activated Ras in turn causes activation of Raf-1 by an undefined mechanism that appears to involve direct interaction of Ras and Raf-1 (*3*, *4*). Activated Raf-1 then phosphorylates and activates MKKs, the specific activators of MAPK (*5*).

Cellular control cannot be achieved by collections of linear signal transduction pathways alone; mechanisms for integrating pathways and generating interactions between pathways are required. MAPK is activated by a diverse set of factors, but notably not by agents that increase intracellular concentrations of cyclic adenosine monophosphate (cAMP) (*6*). Because the antagonism between insulin and cAMP-elevating agents is well known and because cAMP counteracts some biologic effects of oncogenes that activate MAPK (*7*), we conducted studies to determine whether cAMP inhibits the MAPK pathway.

Rat1 fibroblasts that overexpress the human EGFR (Rat1hER) were deprived of serum and incubated with or without forskolin or 3-isobutyl-1-methylxanthine (IBMX) or both agents for 15 min before stimulation with EGF (50 or 200 ng/ml). Forskolin directly activates the catalytic subunit of adenylate cyclase (*8*). IBMX inhibits cAMP phosphodiesterase and may also block inhibitory adenosine receptors linked to G_i to increase the intracellular concentrations of cAMP (*9*). Thus, both agents enhance cAMP accumulation but by different mechanisms. Cells treated with forskolin or IBMX contained activity from the catalytic subunit of cAMP-dependent protein kinase (PKA) [assessed by PKA-inhibitor peptide (PKI)–sensitive histone H1 kinase activity] that was 10 and 4 times that in control cells, respectively (*10*). The stimulation of cells with EGF (50 ng/ml for 5 min) without prior incubation with forskolin or IBMX resulted in PKI-insensitive kinase activity in the cell extract supernatant toward myelin basic protein (MBP) that was four times greater than that in untreated cells (*10*). However, the EGF-dependent increase in MBP kinase ac-

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