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## RAPID COMMUNICATION

### An Inhibitor of Integrin Receptors Blocks Long-Term Potentiation

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A wide variety of cell-cell and cell-matrix interactions are now known to be mediated by members of a large family of heterodimeric transmembrane proteins known as integrins (Ruoslahti & Pierschbacher, 1987). Integrins possess an extracellular receptor site that binds particular matrix components including laminin, fibronectin, vitronectin, and collagens, and an intracellular domain that associates with elements of the membrane cytoskeleton. Given that they play a critical role in forming adhesive contacts in many types of cells, the possibility exists that integrins are also important in the development and maintenance of synaptic contacts. In support of this is the observation that integrin-like proteins are found in the nervous system and participate in the development of anatomical organization. Long-term potentiation (LTP) is associated with changes in the ultrastructure of the synaptic region and possibly the formation of new contacts (Lee, Schottler, Oliver, & Lynch, 1980), effects that might well be expected to involve adhesion proteins including integrins. To explore this idea, we incubated hippocampal slices with the RGDS (Arg-Gly-Asp-Ser) tetrapeptide, a compound that acts as a partial competitive inhibitor of the interactions between integrins and their endogenous ligands (Ruoslahti & Pierschbacher, 1987), and tested for LTP in the Schaffer-commissural projections in field CA1.

Slices were prepared and maintained in an interface chamber (i.e., surface exposed to a moist, oxygenated atmosphere) using methods and techniques described elsewhere. Two to four slices from a single hippocampus were typically tested for LTP. This involved placing two bi-

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polar stimulating electrodes in the Schaffer-commissural projections in the stratum radiatum, with one electrode in field CA1a and the other in CA1c, and a recording pipet in the stratum radiatum of field CA1b. Stimulation currents were adjusted to produce a dendritic field potential of 2–3 mV amplitude and sampling was carried out using pulses delivered once every 20 s. Responses were analyzed for initial slope, amplitude, rise time, decay time, and half-width with a small computer and stored on disk. Recordings were collected until a stable baseline was present for 10 to 20 minutes; if this condition did not occur the slice was not included in the study. After establishing baseline conditions, attempts were made to induce LTP using a theta burst stimulation (TBS) pattern. This paradigm uses short (30 ms) bursts of four pulses with the bursts being repeated five times per second for two seconds (10 bursts). The duration of the stimulation pulses was doubled during TBS. Testing with single pulses was continued for 15–20 min after the effort to induce LTP in one pathway at which point TBS was applied to the second input. Recordings were then collected for an additional 30–45 min.

The results described below were collected from 10 slices (16 pathways total) maintained under control conditions and 22 slices (26 pathways total) exposed to control medium containing 1.0 mM RGDS peptide (Peninsula Laboratories). No evident differences were found in baseline responses in the control and experimental groups. Figure 1 (insert) illustrates a typical baseline response and the measurements collected for analysis. Group means and variances for the potentials recorded immediately prior to TBS are summarized in Table 1. As is evident, equivalent responses were produced by equivalent stimulation currents in the two groups. However, the RGDS peptide did substantially reduce the amount of LTP recorded after TBS. Figure 1 describes typical experiments involving control and experimental conditions. The slice incubated with the RGDS peptide exhibited a robust potentiation immediately after TBS but this decayed back to baseline over the next 15–30 min. The data for the two groups are summarized in Table 1 (bottom) and indicate that the tetrapeptide blocked most but not all of the long-lasting form of LTP.

The above results provide preliminary evidence implicating integrins in the development of long-term potentiation. The RGDS tetrapeptide has a reasonably high affinity for most integrins but provides only a partial block against endogenous ligands; that is, even at high concentrations, RGDS does not completely suppress all binding and hence is sometimes referred to as a partial inhibitor (Gartner & Bennet, 1985). This is of interest in view of the observation that many slices exposed to the tetrapeptide exhibited a small degree of what appeared to be LTP.

Previous work has established that LTP in field CA1 is accompanied by structural changes in dendritic spines and possibly by the formation

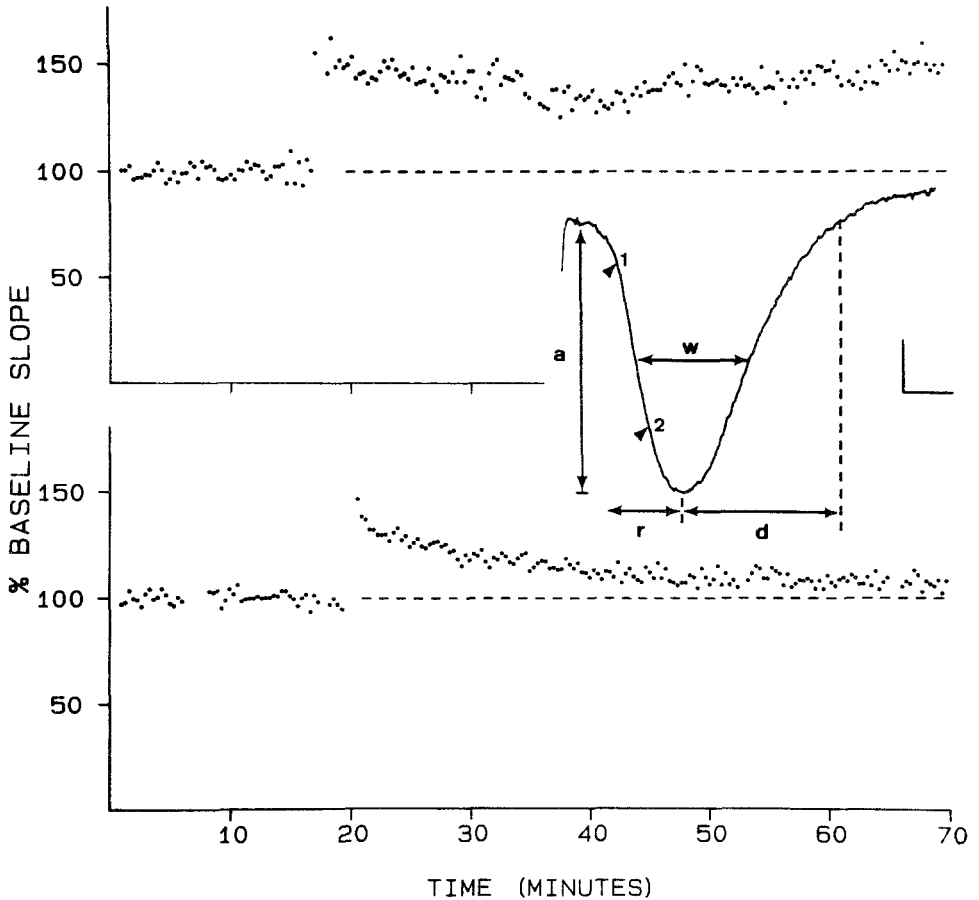


FIG. 1. Effects of theta burst stimulation (TBS) on two slices, one maintained in control medium (upper graph) and the other in control medium plus 1.0 mM RGDS (lower graph). The insert is an evoked response taken from the control slice immediately prior to TBS and illustrates the measurements used in Table 1. (a) amplitude, (r) rise time, (d) decay time, (w) half width, (1 and 2) initial slope. The calibration bars denote 0.5 mV and 2.5 ms.

of new contacts (Lee et al., 1980). These events presumably involve disassembly and reassembly of the cytoskeleton. Integrins are thought to regulate the organization of the cytoskeleton and to anchor it in particular configurations via their contacts with extracellular matrix proteins and with other cells. We have previously proposed that a calcium-activated protease (calpain) that cleaves membrane cytoskeleton proteins including spectrin is involved in the initial stages of cytoskeletal reorganization (Lynch & Baudry, 1984). This could serve to release the spine and/or local patches of membrane from the constraints imposed by in-

TABLE 1  
Effects of the RGDS Tetra Peptide on Baseline Responses and Magnitude of LTP Produced by Theta Burst Stimulation

		Baseline response characteristic ( $\bar{x} \pm SD$ )					
	Stim. current	Slope	Amplitude	Half rise time	Half decay time	Half width	
		(mV/msec)	(mV)	(msec)	(msec)	(msec)	
Control ( $n = 16$ )	26.1 $\pm$ 10.3 (microamps)	0.98 $\pm$ .22	2.13 $\pm$ .48	2.16 $\pm$ .53	3.21 $\pm$ .55	5.01 $\pm$ .75	
RGDS ( $n = 26$ )	24.7 $\pm$ 20.5	0.85 $\pm$ .27	2.13 $\pm$ .59	2.54 $\pm$ .50	3.21 $\pm$ .54	5.16 $\pm$ .72	
Percentage increase above baseline ( $\bar{x} \pm SD$ )							
	5 min	10 min	15 min	20 min	30 min		
Control ( $n = 16$ )	162 $\pm$ 13	152 $\pm$ 14	149 $\pm$ 13	147 $\pm$ 14	147 $\pm$ 14	147 $\pm$ 14	
RGDS ( $n = 26$ )	144 $\pm$ 20*	131 $\pm$ 16*	125 $\pm$ 16*	123 $\pm$ 15*	118 $\pm$ 19*		

\*  $p < .01$  (two-tailed  $t$  test).

tegrins and allow for new shapes (or contacts) to emerge. The binding of newly exposed integrins to extracellular proteins could then serve as a trigger for the reassembly and restabilization of the cytoskeleton; events similar to this are thought to be involved in platelet aggregation (Baldassare, Bakshian, Knipp, & Fisher, 1985). Evaluation of this speculative hypothesis requires further work on the physiological effects of RGD-containing peptides and biochemical studies on the existence and nature of synaptic integrins.

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