3-h-period at 20 °C was filtered on a Buchner funnel, washed twice with ethyl ether and dried. This dried material was extracted overnight with 200 ml of 70% (v/v)EtOH, and the supernatant obtained by centrifugation was evaporated to dryness under reduced pressure at 30–35 °C (step 3).

This residue was successively extracted with 33 ml of 0.1 M ammonium acetate buffer (pH5) and the supernatant collected was fractionated on a Sephadex G-25 fine column (figure 1). The active fraction (shaded area) was lyophilized (step 4). An amount of this material (corresponding to 800 ml of initial colostrum) was chromatographed on a preparative peptide-analyzer by employing Aminex A-5 (Bio-Rad Laboratories) under the conditions described in figure 2. The separation pattern (ninhydrin test after alkaline hydrolysis according to Moore and Stein[8] in order to avoid interference due to eluent buffers) was recorded at 570 nm. The active fraction was lyophilized (step 5). The fractions obtained at the points step 2, 3, 4 an 5 have been tested biologically, and the results attained listed in table 1 with the respective values of their dried weights and proteic contents. The fraction of the step 5 was moreover submitted to the enzymatic treatments described and then tested for its biological activity on rat uterus preparations. The results obtained are listed in table 2.

Table 2. Enzymatic digestion of a partially purified colostrokinin*

Sample	Per cent activity**
Control	100
Pepsin digested	100
Papain digested	2– 3
Trypsin digested	38–42

*Biological activity on rat uterus in vitro. **Per cent residual activity referred to control considered = 100.

Discussion. The steps of purification of colostrokinin now proposed only partially overlap those described by Yamazaki and Moriya[5]. The short time of enzyme-substrate contact was selected according to previous results by Beretta and Ormas[9]. The 2 successive acetic acid extractions (2 N and glacial) allow a good separation of the kinin from the high molecular weight compounds (i.e. proteins, nucleic acids, etc.) while the precipitation with an excess of ethyl ether removes the lipids still present in the menstruum. The elution Kd from the Sephadex G-25 column suggests that the active fraction is characterized by a mol.wt between 1000 and 2000. The successive purification on Aminex A-5 reveals its basic properties. This last finding is confirmed by the lack of biological activity showed by the papain-treated preparations (table 2). Since the activity of the kinin has been not damaged by pepsin and only partially by trypsin (enzymes present in the animal gastrointestinal tract), it can be suggested that when released by salivary kallikrein, colostrokinin may pass uninjured through the gastric environment and be hydrolyzed only within the duodenal tract, where, however, trypsin might exert not only a kininase-like but also a kininogenase-like activity[4]. On account of the purification factors calculated, this new procedure appears to be simple and rapid enough to allow the preparation of relatively large amounts of the kinin containing material. Studies on its chemical and biological properties are now in process in our laboratories.

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Pentobarbital: Presynaptic effect in the squid giant synapse[1]

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Summary. In concentrations that produced synaptic blockade in the squid giant synapse, sodium pentobarbital produced a dose-related, reversible decrease of the 'calcium spike' of the presynaptic terminal.

Barbiturates block synaptic transmission at lower concentrations than they block conduction along nerves[4–6]. These lower concentrations are more relevant to the general anesthetic concentrations in man, and the postsynaptic membrane is affected in this concentration range[7–9]. There has been considerable debate, however, as to whether barbiturates also have presynaptic effects. For example, a barbiturate-induced decrease in quantal content has been demonstrated in cat motoneurons[10], and barbiturates have been shown to inhibit depolarization-induced calcium uptake in rat synaptosomes[11] and in symptathetic ganglia[12]; but barbiturates cause no change or an increase in quantal content at the frog neuromuscular junction[7,13]. There have been few intracellular studies of direct presynaptic effects of barbiturates because of the difficulty of working with presynaptic terminals. The squid giant synapse is unusual in that both the pre- and the postsynaptic terminals can be

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penetrated with microelectrodes with relative ease. Therefore, in order to test whether there are barbiturate-induced presynaptic changes, we studied the effects of pentobarbital on this preparation.

Methods. Standard dissection and intracellular microelectrode recording techniques were used[14,15]. Experiments were performed on isolated stellate ganglia from Loligo pealeii at 17–18 °C and at pH 6.5 unless otherwise mentioned. In order to measure suprathreshold postsynaptic potentials (PSP's) hyperpolazizing current pulses were injected through a second microelectrode near the postsynaptic recording electrode. We recorded the 'calcium spike' using a technique similar to that of Katz and Miledi[16]. The sodium currents were blocked by 2×10^{-7} g/ml tetrodotoxin, and potassium currents diminished by 5 mM 4-aminopyridine. The latter compound is effective on extracellular application in blocking potassium currents, allowing the recording of the calcium spike[17,18].

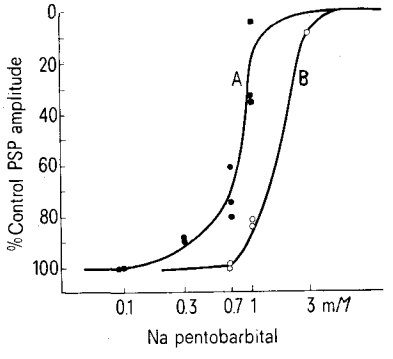

Fig. 1. Percentage of control PSP amplitude at increasing concentrations of sodium pentobarbital at 2 pH's. *A* pH 6.5; *B* pH 8.0.

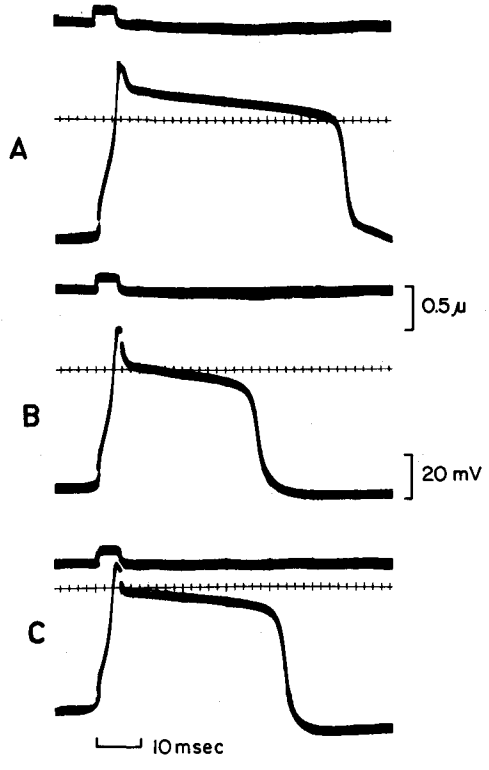

Fig. 2. Effect of pentobarbital on the calcium spike. In each of the 3 records the top trace is current, the bottom trace is presynaptic membrane potential. *A* control; *B* after equilibration in 0.7 mM pentobarbital for 110 min; *C* partial reversal.

Results and discussion. In order to find the concentration range in which synaptic transmission is affected by pentobarbital in the giant synapse, we measured PSP amplitudes in the presence of increasing concentrations of the drug in normal artificial seawater. Curve A in figure 1 shows the dose-response relationship for the effect of pentobarbital on PSP amplitude at pH 6.5. Pentobarbital had significantly ($p < 0.05$) less effect at pH 8.0. This indicates either that the drug, which is a weak acid, is more potent in the uncharged form; or, that the drug has an internal site of action. This pH dependence of the barbiturates' synaptic effect has not been seen previously[19]. In this concentration range there were no significant drug-related changes in postsynaptic critical membrane potential or in pre- or postsynaptic resting membrane potential or action potential amplitude. Next, we looked for pentobarbital-induced changes in presynaptic function in the same concentration range. Since the conduction of the action potential into the presynaptic terminal was unaffected, a presynaptic effect of pentobarbital would have to be in the excitation-secretion coupling process. Katz and Miledi have recorded a calcium-dependent, regenerative spike in the presynaptic terminal of the squid synapse. This spike is presumed to reflect an inward calcium current associated with excitation-secretion coupling[16].

With sodium and potassium currents blocked, we recorded this 'calcium spike' in the presence of either 0.3, 0.7 or 1.0 mM pentobarbital in 6 preparations; figure 2 shows the effect at 0.7 mM. There was no change in the amount of current needed to trigger the calcium spike; but the drug decreased the plateau duration and amplitude. Concentrations from 0.3 to 1.0 mM reduced the duration of the calcium spike by 16–86%, respectively. The same concentrations reduced the amplitude of the plateau by 5–36%. With few exceptions, the calcium spike returned toward control size upon washing away the drug. Since the degree of potassium blockade by 4-aminopyridine in the squid synapse is unknown we must consider the possibility that pentobarbital is decreasing the size of the 'calcium spike' by increasing potassium conductance. However, barbiturates appear to have no effect or to decrease potassium conductances[7,9,20], but they can decrease calcium fluxes[11,12]. Our results are best explained by a decrease in the voltage dependent influx of calcium by pentobarbital.

These results demonstrate that barbiturates can affect presynaptic function in the concentration range that blocks synaptic transmission. More experimental work will be required to determine the relative contribution of this presynaptic effect of the barbiturates to the induced synaptic block. In some synapses it may be a dominant effect while in others it may play a modulating role or be entirely absent. The pentobarbital-induced change in the 'calcium spike' suggests an effect on excitation-secretion coupling. This is the first report that a drug, other than multivalent ions[16,21], has been shown to directly affect the 'calcium spike' of the squid presynaptic terminal.

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