stem neurons likewise has revealed both an inhibitory and an activating action of these substances when applied by microiontophoresis [6]. Naloxone blocked the above-mentioned effects of endogenous morphine-like compounds. Davies and Dray [6], in particular, attribute differences in the sensitivity of neurons to the possibility that different opiate receptors may exist in the CNS. Heterogeneous changes in unit activity are also observed during electrical stimulation of the raphe nuclei [1], which is known [8] to cause analgesia in animals, possibly on account of an increase in the concentration of endogenous morphine-like peptides in the CNS.

It can thus be tentatively suggested that analgesia connected with an increase in the level of endogenous morphine-like peptides in the CNS following stimulation of the raphe nuclei and also following injection of enkephalins or preparations of the morphine group from an outside source, is accompanied by disturbances of functional relations between neurons of both subcortical and cortical formations. This hypothesis is supported by data showing that sensomotor cortical cells participate in responses to nociceptive stimulation [11].

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THE ROLE OF CALCIUM IN THE MECHANISM OF CHANGES IN VASCULAR REACTIVITY DUE TO RESERPINE

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Under the influence of reserpine there is a marked decrease in the calcium concentration in segments of the distal aorta and femoral arteries of rabbits, incubated in salt solution. These changes develop parallel with changes in the character of responses of the vascular segments to direct electrical stimulation (weakening of the effects of contraction, followed by relaxation). A decrease in the calcium concentration in the vascular wall is suggested as one cause of the change in vascular reactivity.

KEY WORDS: calcium; reserpine; reactivity of blood vessels.

It has been concluded from experimental data obtained during a study of the effect of reserpine on neurogenic vasomotor responses that the fall in blood pressure is due to modified vascular reactivity to nervous influences [1, 4, 5]. Changes in the reactive properties of the blood vessel wall under the influence of reserpine, manifested as the replacement of contraction by relaxation, have been confirmed by recording contractile responses of isolated vascular segments to direct, measured electrical stimulation [3]. Reserpine is a sympatholytic drug which causes exhaustion of the tissue noradrenalin reserves [13]. However, this

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TABLE 1. Calcium Content in Wall of Blood Vessels (in meq/kg dry weight) after Incubation in Salt Solution Containing Reserpine ($1 \cdot 10^{-4}$ g/ml)

Incubation condition	Experiment number																				Mean value	Significance of differences
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Incubation for 20 min in salt solution	10,0	12,2	5,1	5,1	16,5	7,5	10,0	7,0	3,35	2,0	5,4	2,25	2,0	5,0	6,0	7,5	5,75	8,0	14,0	10,0	7,23±0,88 (I)	
Incubation in salt solution with resperine:																						
20 min	4,75	3,25	5,0	3,9	5,0	2,0	8,0	3,0	5,0	2,5	1,1	1,7	1,0	4,0	3,6	3,2	3,2	3,0	2,5	7,5	3,71±0,42 (II)	P_{II-III}, $P_{II-IV} < 0{,}001$, $P_{II-III} < 0{,}01$
2 h	4,0	3,0	2,0	1,5	2,0	1,5	4,0	2,6	1,5	2,2	0,6	0,5	0,8	0,6	2,0	0,6	1,0	1,6	2,5	1,5	1,81±0,24 (III)	P_{I-III}, $P_{III-IV} < 0{,}001$
3 h	3,8	2,2	1,6	0,2	0,2	0,6	1,7	2,0	0,5	1,1	0,2	0,2	0,2	0,2	0,3	0,5	0,2	0,3	1,5	0,5	0,92±0,18 (IV)	$P_{I-IV} < 0{,}001$

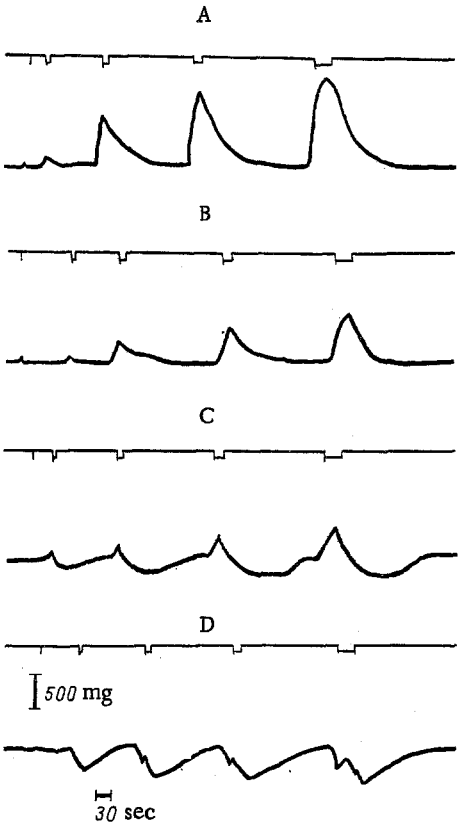

Fig. 1. Changes in responses of segment of rabbit femoral artery to direct electrical stimulation under influence of reserpine. A) Initial responses, B, C, D) 20 min, 2 h, and 3 h respectively after addition of $1 \cdot 10^{-4}$ g/ml reserpine to incubation medium. Here and in Figs. 2 and 3, from top to bottom: marker of stimulation (20 Hz, 20 V, 20 msec), contractions of vascular segment.

aspect of the action of reserpine cannot be completely responsibe for such profound changes in the reactive properties of the vascular wall, for they are also characteristic of vessels which have no innervation; according to Fleisch [8], this includes the rat aorta. The determining role of noradrenalin for changes in vascular reactivity is also ruled out by the fact that during electrical stimulation the effect is only partially due to liberated noradrenalin, and it is predominantly connected with the direct action of the electric current of the cell membrane and on the mechanisms of coupling of excitation and contraction [7]. The change in the character of responses under the influence of reserpine must therefore be due primarily to its effect on these phenomena. Despite intensive research, the mechanisms controlling contraction and relaxation of vascular smooth muscle are not yet clear. However, there is evidence that intracellular calcium regulates both the beginning of contraction and the degree of relaxation of the vessels [14]. Contraction of the vessels depends on factors influencing the transmembrane calcium inflow [11]. In the opinion of some workers, the final common path for the action of dilators is a decrease in the intracellular calcium concentration [10]. Since a decrease in the calcium concentration has been found in the vessel wall in reserpinized animals [6], it was interesting to compare the changes discovered in the responses of vascular segments to electrical stimulation with the calcium concentration in the vascular wall at different stages of the change in reactivity under the influence of reserpine.

EXPERIMENTAL METHOD

Responses of segments of the distal aorta and femoral arteries of rabbits to quantitatively measured electrical stimulation of the vascular wall [2] were recorded by means of a

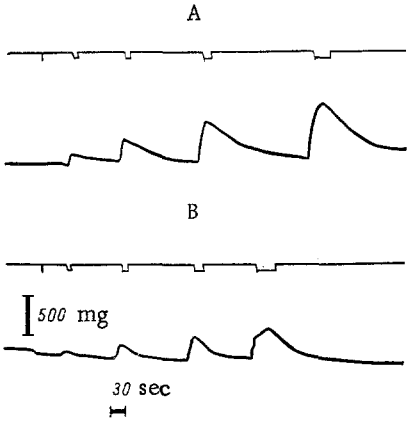

Fig. 2

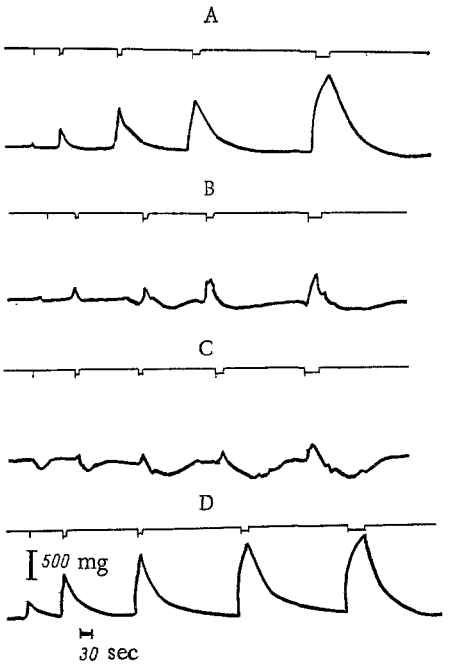

Fig. 3

Fig. 2. Effect of incubation of segment of rabbit femoral artery for 2 h in calcium-free solution on character of responses to electrical stimulation. A) Initial responses; B) after incubation in calcium-free solution.

Fig. 3. Responses of distal segment of rabbit aorta to direct electrical stimulation. A) Intial responses; B) after incubation of vascular segments for 1.5 h in solution containing $1 \cdot 10^{-4}$ g/ml reserpine; C) after replacement of incubation medium by fresh salt solution; D) after subsequent incubation of segment for 24 h in salt solution.

type 6MKh1S mechanotron. Changes in the character of the responses were recorded 20 min and 2 and 3 h after addition of reserpine in concentrations of $5 \cdot 10^{-5}$ to 10^{-4} g/ml to the solution in the muscle chamber, and they were compared with the calcium concentration in the vascular tissue determined at the same time. To determine calcium the tissue was dried, then ignited, and the residue was dissolved in concentrated nitric acid and titrated with EGTA, using a PHM-62 (Denmark) pH-meter, equipped with an automatic titration unit, and working under pH-stat conditions. The calcium concentration in the tissue was expressed in meq/kg dry weight.

EXPERIMENTAL RESULTS

Under the influence of reserpine in a concentration of $5 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ g/ml the contractible responses of the vascular segments to electrical stimulation of varied duration initially became weaker (Fig. 1A, B). This weakening of contraction became more apparent as the duration of incubation of the preparations in the solution containing reserpine increased. The response then became biphasic: An initial slight contraction was followed by relaxation. An effect of this sort, recorded 2 h after addition of reserpine, is illustrated in Fig. 1C. Gradually the phase of the contraction disappeared and was replaced by relaxation, which increased in intensity even after the end of stimulation (Fig. 1D).

The total calcium concentration in the tissue of the aorta and femoral arteries of 20 rabbits before incubation averaged 7.23 meq/kg dry weight (Table 1), without any significant differences along the length of the aorta. After incubation for 20 min in solution with reserpine the calcium content was reduced by 49%, and after 2 and 3 h by 75.1 and 87.3% respectively below the initial level. In control experiments the calcium concentration in vascular tissue incubated for 20 min and 2 and 3 h in salt solution without reserpine was almost indistinguishable from the initial level.

With an increase in the duration of exposure to reserpine the calcium content in the walls of the blood vessels thus fell statistically significantly, parallel with the change in the character of the vascular responses to electrical stimulation, of which it was evidently the main cause. This suggestion was confirmed by the participation of the tissue calcium in the mechanism of the contraction effects when preliminary incubation of the vascular segments for 2 h in calcium-free solution (causing a decrease in the calcium concentration in the tissue) led to marked weakening of the contractile effects to unchanged stimulation in ordinary salt solution (Fig. 2A and B).

It is an interesting fact in connection with the problem of the role of calcium in the development of vasomotor effects that during prolonged recording of the changes in the responses of the vascular segments to electrical stimulation in the presence of reserpine the transition from contraction to relaxation occurred much more rapidly if the incubation medium was replaced by fresh salt solution (Fig. 3B and C). This phenomenon can be understood if it is remembered that by replacing the solution the excess of calcium formed in it through liberation of this cation from the reserpinized tissue was removed. Conversely, if the vascular segment, when the character of its response to electrical stimulation had been changed under the influence of reserpine, was left in salt solution for 24 h, the initial reactivity was restored (compare Fig. 3C and Fig. 3D and A). The view that the effects of reserpine are connected with changes in the calcium concentration is supported by results showing that, by increasing the calcium concentration in the solution, it is possible to counteract the blocking effect of reserpine on contractile responses of isolated preparations of the guinea pig heart [12] and uterus [17].

In these experiments a progressive decrease in the calcium content in the vascular wall under the influence of reserpine was followed by changes not only in the amplitude but also in the functional direction of the vasomotor responses (contraction was replaced by relaxation). However, this does not mean that the relaxation response developed in the absence of calcium. Calcium is essential for both contraction and relaxation: Both types of response, when evoked by different concentrations of isoproterenol, are abolished during incubation of the vascular segments in calcium-free solution [18]. During excitation there is an increase in the free intracellular calcium on account of liberation from intracellular storage sites and an influx from the extracellular space [16]. Relaxation of smooth muscles is accompanied by the outflow of calcium from the cells, by its binding in the sarcoplasmic reticulum, and by a decrease in its supply to contractile proteins [9, 15]. These calcium displacements take place during the reaction itself, which they serve. Reserpine, however, by reducing the total calcium content in the tissue and its spatial distribution in the cell, evidently creates conditions favoring relaxation. Special investigations will be carried out to study how changes in the tissue calcium concentration help to predetermine the character of the vascular responses to stimulation.

Comparison of changes in the calcium concentration in the walls of blood vessels with changes in their reactivity was made in the present experiments on isolated vascular segments. However, the agreement between changes taking place under the influence of reserpine in the responses of blood vessels to nervous excitation and to electrical stimulation of vascular segments [5] suggests that the same causes are responsible for changes in reactivity in the intact organism.

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BRADYKININ AS A FACTOR REGULATING SYNAPTIC INPUT TO *Helix pomatia* NEURONS

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The effect of bradykinin on spontaneous unit activity was studied in the subesophageal ganglion of *Helix pomatia* by microiontophoresis. Bradykinin was shown not only to facilitate unit responses to synaptic activation, but also to prolonged action potential generation evoked by this activation. The effects of bradykinin were observed in experiments carried out during the spring.

KEY WORDS: bradykinin; neuronal oligopeptides; synaptic transmission.

An important role in the mechanisms of chemical interaction between neurons is played not only by the classical mediators (acetylcholine, noradrenalin, dopamine, serotonin), and amino acids, but also by certain oligopeptides (substance P, vasopressin, oxytocin, releasing factors). These peptides, it is held, can act in the CNS as mediators or modulators [1, 11]. It accordingly seemed very likely that these oligopeptides can actively influence the performance of their integrative functions by nerve cells [3, 12].

Sensitivity to physiologically active peptides such as oxytocin, vasopressin, angiotensin-II, and physalaemin has been discovered in molluscan neurons [3, 5, 12]. Several peptide factors which significantly change the physiological properties of the neuron in these animals have been isolated from an extract of the nerve tissue of mollusks [2, 10]. These findings indicate that molluscan neurons can be used as a model with which to study the mechanisms of action of oligopeptides on single neurons and to investigate interaction between these substances and other biologically active compounds in the formation of the integrative functions of central neurons.

In this investigation the effect of bradykinin, and endogenous oligopeptide inducing pain in man and animals on systemic injection [7-9], on spontaneous unit activity was studied in the subesophageal ganglion of *Helix pomatia*.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated circumesophageal nerve ring, placed in hemolymph, and a semi-intact preparation of the mollusk with nervous connections undamaged. The use of the semi-intact preparation enabled perfusion to be carried out with standard physiological saline for mollusks, pH 7.6. After removal of the membrane, a microelectrode

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