temperature, although the grain locations were always indicative of nerve ending labeling.

These results show that increased reactivity of the contents of certain types of brain synaptic vesicles toward OsO_4 can be obtained by performing the final fixation step at elevated temperatures. Although the product formed resembles superficially the granularity described within synaptic vesicles after $KMnO_4$ fixation, variation in catecholamine content occurs without detectable alteration in the appearance of the present osmiophilic substance. Moreover, the process which permits the visualization of this material actually seems to extract the catecholamine radioactivity. Thus, while granular synaptic vesicles may be an index to monoamine-storage, the granular material demonstrated by exposure to warm OsO_4 does not seem to be the monoamine itself. If the warming procedure facilitates the ability of OsO_4 to react with vesicle contents by increasing its potency as an oxidizing agent, then possibly other reducing substances[13] known to be present within the hypothalamus and related to monoamine neurons, such as ascorbic acid, could be responsible for the reaction observed here. The exact cause for the granular deposits developed by $KMnO_4$ fixation within presumed monoamine-containing nerves and its relation to the osmiophilic substance described here must now be investigated[14].

Zusammenfassung. Nach Fixierung durch Glutaraldehyddurchströmung und Behandlung mit OsO_4 bei 60 °C während 30 min erhält man im Rattenhirn elektronendichte Niederschläge innerhalb der kleinen synaptischen Vesikel. Amin-Entleerungsversuche und autoradiographische Untersuchungen haben gezeigt, dass diese Niederschläge nicht mit dem Katecholamingehalt des Gehirns zusammenhängen.

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Triphasic Intestinal Reaction on Adrenaline in the Rat

According to Ahlquist[1], Levy[2] and Furchgott[3] both canine ileum and rabbit duodenum relax after stimulation of either α- or β-receptors. Kawai[4,5], however, has found a contraction of guinea-pig ileum (maximal in the terminal part) after adrenaline (A). The present experiments in vitro were undertaken to analyse the effects of A upon rat intestinal segments.

Results. Adrenaline in concentrations of $5 \times 10^{-9} M$ to $1 \times 10^{-7} M$ caused relaxation and inhibited peristalsis in proportion to the amount of A in the bath. It is clearly evident that concentrations of $5 \times 10^{-7} M$ to $5 \times 10^{-6} M$ and sometimes $1 \times 10^{-5} M$ caused a triphasic change in the tone (Figure 1). After relaxation there followed an increase of the tone, which reached or overran the previous level, and was succeeded by a secondary relaxation. Atropine added to the bath fluid in concentration of 1 mcg/ml did not change these effects. The intensity of contractile phase was lower in duodenal segments and was increased in terminal parts of intestinal segments. The triphasic reaction to A in concentrations of $5 \times 10^{-7} M$ to $1 \times 10^{-5} M$ was also observed in rabbit terminal ileum and in human processus vermiformis tissue.

When propranolol in concentrations of $5 \times 10^{-5} M$ to $1 \times 10^{-4} M$ was added to the bath approximately 5 min before A, the relaxation of rat's intestine was prevented and the increase of tone was evidently augmented (compare 2 in Figure 1). Phentolamine in concentration of 5 mcg/ml inhibited the increase of tone observed after A, but either did not change or even increased the degree of relaxation (compare 3 in Figure 1 and C in Figure 2). The reaction of rat's intestine to high doses of A after phentolamine was similar to that observed after low doses of A. When both α- and β-blocking drugs were added to the bath concomitantly, A effects were completely prevented in concentrations up to $2 \times 10^{-5} M$. Methysergide maleate (deseril) added to the bath in concentration of $1–3 \times 10^{-3} M$ decreased slightly the intensity of triphasic reaction to A but did not change its character (B in Figure 2). Isoproterenol in concentration of $5 \times 10^{-9} M$ to $1 \times 10^{-6} M$ caused relaxation and disturbed peristaltic movements of rat's intestine and duodenum. Isoproterenol did not cause the contraction of intestinal smooth muscles, even in highest concentrations applied. The effects of isoproterenol were completely abolished by propranolol in concentrations of $5 \times 10^{-5} M$ to $1 \times 10^{-4} M$.

Since both isoproterenol and A applied in low concentrations caused relaxation of intestine, which was prevented by propranolol, it is evident that their action concerns β-receptors. However, the contractile phase of the reactions of intestine to high doses of A may be due to the stimulation of α-receptors, since it is prevented by phentolamine. The contractile phase of A effects does not change in the presence of propranolol, pointing to the same conclusion: adrenaline causes contraction and not relaxation of intestinal smooth muscles, exerting its influence by stimulation of α-receptors. Contractions of the gastrointestinal smooth muscles following α-receptor stimulation have been observed in muscle of the biliary tree of the guinea-pig by Crema et al.[6], and in both the

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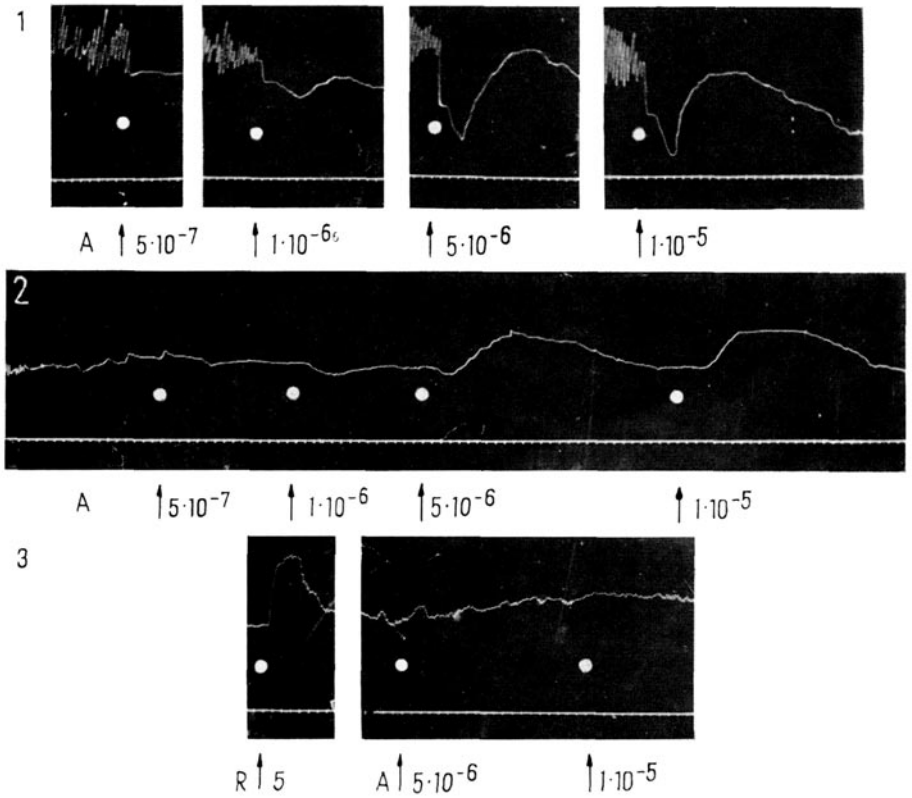

Fig. 1. Influence of propranolol and phentolamine on the action of adrenaline on rat's ileum in vitro. The action of adrenaline (in molar concentrations) before antagonists (1), after addition to the bath of propranolol in the concentration of $5 \times 10^{-5} M$ (2) and after phentolamine in concentration of 5 mcg/ml (3). A, adrenaline; R, phentolamine.

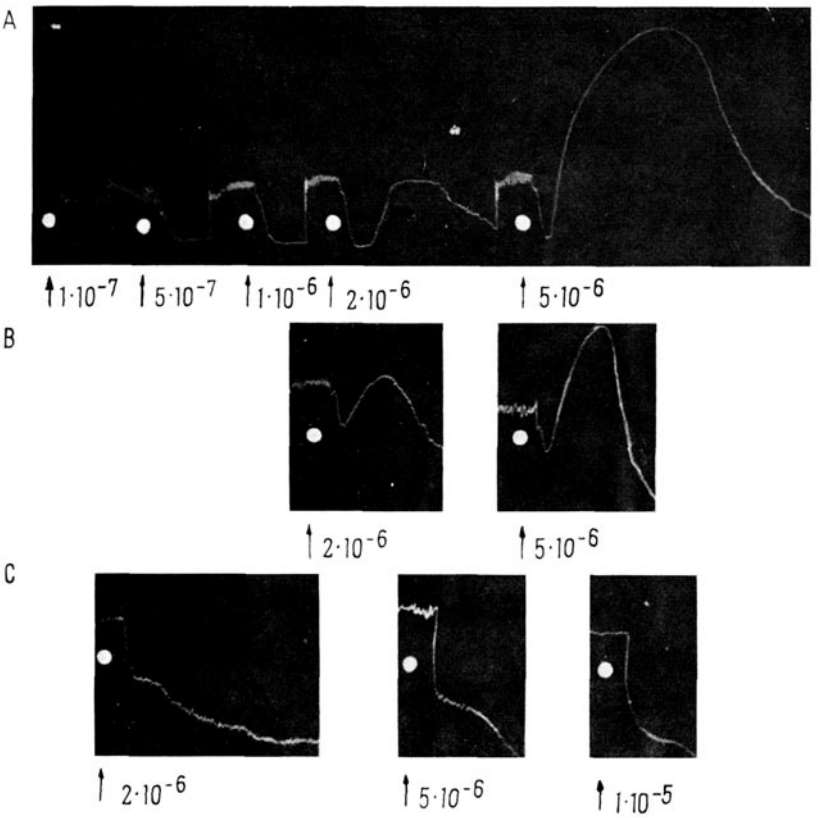

Fig. 2. Effect of methysergide maleate and phentolamine on the action of adrenaline on rat's ileum in vitro. The action of adrenaline (in molar concentrations) before antagonists (A), after methysergide maleate in concentration of $3 \times 10^{-8} M$ (B) or phentolamine in concentration of 5 mcg/ml (C) were added to the bath.

terminal ileum[7,8] and œsophagus[9] of the guinea-pig. Recently, CHRISTENSEN and DANIEL[10] observed that noradrenaline produced contractions of the strips from the lowest centimeter of the cat oesophagus. This contractile response was opposed by tolazoline and atropine but not by propranolol. These authors were of opinion that adrenergic α-receptors in the cat oesophagus are excitatory. Since according to LANDS et al.[11] both types of catecholamine receptor (that is α- and β-receptors) are present in intestine, low doses of A stimulate either β-receptors only or both α- and β-receptors. In the latter case it is possible that the ratio of α- and β-receptors distribution in various segments of intestine is responsible for the different degrees of relaxation of smooth muscles due to general prevailing amount of β-receptors against α-receptors in intestine and their varying distribution. Further experiments to elucidate the phenomenon observed are in progress.

Zusammenfassung. Adrenalin bewirkt eine triphasische Reaktion am isolierten Rattenileum. Die Kontraktionsphase beruht wahrscheinlich auf Stimulierung der α-Rezeptoren.

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Immunopharmacologic Activity of 1-Aminocyclopentane-1-Carboxylic Acid

1-Aminocyclopentane-1-carboxylic acid (ACPC) is a non-metabolizable, unnatural amino acid lacking an α-hydrogen atom. First synthesized in 1911[1], it was not tested for biological activity until the 1950's, when it was reported to inhibit growth of various experimental tumors and be actively concentrated in malignant cells[2–4]. With the exception of some activity in multiple myeloma, ACPC has been essentially ineffective as an antitumor agent in humans[5]. Its mechanism of action is unknown despite intensive investigation. Aminoaciduria results after oral administration in man, but loss of amino acids is probably not responsible for antitumor activity[6]. ACPC is inactive as an amino acid antagonist in bacterial systems; however, it antagonizes incorporation of valine into proteins in the rat, possibly by preventing attachment of valine to S-RNA[7]. The toxic effects of ACPC can be prevented in chickens by valine[8] and in *Escherichia coli* by methionine and valine[9]. One of the initial signs of toxicity is body weight loss, which can be prevented by force-feeding[10]. Other studies show that ACPC does not inhibit enzymatic oxidation or transamination of amino acids[11] and is not incorporated into protein[12]. The long half-life of ACPC, 20–30 days in rodents and 2–3 days in monkeys and humans[13] is probably due to extensive renal reabsorption and lack of metabolism. These biological characteristics have led to its use as a model for the study of amino acid transport[14].

The immunosuppressive properties of ACPC became evident through our observation that it could suppress experimental allergic encephalomyelitis (EAE) in rats in a dose-related manner[15] (Figure 1). This laboratory-induced immunopathy, a prototype syndrome of cellular hypersensitivity reactions, is useful for detecting and evaluating immunosuppressive agents[16,17]. In this procedure, animals are evaluated for gross hind limb paralysis 14 days after administration of an encephalitogenic emulsion in the hind foot-pad[16]. Study of a series of ACPC analogs indicated that the 5-membered ring is essential for immunosuppressive activity in EAE. Alkylamino substitutions greater than methyl decrease activity. In addition to preventing paralysis, ACPC prevents or suppresses other sequela of EAE, such as reduction of body and stress organ weights, hematologic changes, paw swelling and histopathologic lesions of the brain and spinal cord. Methionine at 5 times and valine and leucine each at 28 times the dose of ACPC failed to prevent its immunosuppressive action in EAE. ACPC likewise suppressed EAE in rabbits and guinea-pigs. At similar doses it is effective in other models of cellular hypersensitivity; for example, it reduces a dinitrochlorobenzene-induced contact dermatitis in guinea-pigs and significantly increases the rejection time of skin grafts in mice (BALB/C to CBA/2).

ACPC is also effective in decreasing circulating antibody responses, including the primary type obtained in the mouse by injecting sheep red blood cells or *Salmonella*

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