The heptane extract of the apparent lactam metabolite was subjected to thin-layer chromatography on silica gel in several solvent systems. Only one radioactive spot was present, which corresponded in Rf value to that of the authentic lactam (Table). The radioactive material was eluted and subjected to preparative gas chromatography at 180 °C in a Perkin-Elmer gas chromatograph, equipped with 6 foot glass columns packed with 1% QF-1 on Gas Chrom Q and stream-splitter (80:20). A major peak was observed with a retention time of 2.4 min, the same as that of authentic lactam. Eluates from the column were trapped in Pasteur pipets and the contents counted. Only the peak corresponding in retention time with that of the lactam metabolite (2.4 min) contained significant radioactivity.

Rats given prolintane-^{14}C (10 mg/kg, i.v.) excreted approximately 40% of the dose in the urine in 24 h but only traces, if any as the lactam metabolite. The drug itself was almost completely metabolized.

Thin-layer chromatography of the metabolite and authetic 1-(α-propylphenethyl)-pyrrolidin-2-one

Solvent system	Metabolite	Synthetic compound
1	0.60	0.60
2	0.44	0.44
3	0.58	0.58

Glass plates coated with silica gel G, 250 μm. Compounds were detected by radiometric scanning and Dragendorf's reagent. Solvent systems were prepared as follows: 1. benzene-dioxane-ammonia 60:35:5[6]; 2. cyclohexane-diethylaminebenzene 75:20:15[7]; benzene-ethanol-12 *N* ammonia 95:15:5[7].

In conclusion, the present study describes a further example of metabolic conversion of a substituted pyrrolidine to the corresponding lactam. Since the lactam may, potentially, at least, be converted to the open-chain amino acid, the reaction is properly classified as a type of N-dealkylation. Information on the mechanisms of drug metabolism reactions may have, in addition to its theoretical value, important pharmacologic application, since the various intermediate formed may themselves posses biologic activity.

Zusammenfassung. Prolintan, N-1-(α-Benzylbutyl)-Pyrrolidin, wurde in Kaninchen durch ein Mikrosomensystem der Leber in das entsprechende Laktam, N-1-(α-Benzylbutyl)-Pyrrolidin-2-on, umgebaut. Das gleiche Stoffwechselprodukt fand sich auch im Gewebe von Ratten, denen das Medikament gegeben wurde; allenfalls Spuren davon wurden im Urin der Ratten ausgeschieden.

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A Note of the Dual Effect of Prostaglandin E_1 on the Responses of the Guinea-Pig Vas Deferens to Nerve Stimulation

Divergent results have been obtained concerning the effects of prostaglandins on the motor response of the guinea-pig vas deferens to nerve stimulation. Mantegazza and Naimzada[1] reported that high concentrations of PGE_1 potentiated this response. Sjöstrand and Swedin[2] noted that PGE_1 usually caused an inhibition but sometimes a potentiation of the response. Euler and Hedqvist[3] observed that low concentrations of PGE_1 and PGE_2 inhibited the response, while it was enhanced by higher concentrations. Ambache and Zar[4] found that low concentrations of PGE_2 inhibited the response to low frequency stimulation but rather potentiated the response to high frequency stimulation. In order to obtain a somewhat more detailed information on the action of PGE_1 on the response to nerve stimulation of the guinea-pig vas deferens, a study with combined electrical and mechanical registration has been performed.

Material and methods. For electrical recording the sucrose gap technique was used. The preparation, sucrose gap apparatus with stimulating electrodes, solutions, temperature and other conditions were essentially the same as those used by Burnstock et al.[5]. Isometric tension was recorded with a strain gauge transducer. Conventional amplifiers and a Hellige Helcoscriptor (He-86) were used. Nerve stimulation with square wave pulses of 0.5 msec duration and a voltage of 10–40 V was applied by a Grass S4 stimulator. The report is based on observations on 14 vasa deferentia from 10 guinea-pigs weighing about 500 g.

Results. PGE_1 in concentrations of 10–200 ng/ml caused a depolarization of the smooth muscle of the vas deferens. The depolarization was usually moderate as in Figure 1a. It increased in amplitude with increasing PGE_1 concentration but was never large enough to reach the threshold for firing of action potentials (AP). However, there were rather large differences in sensitivity among the preparations.

PGE_1 decreased the magnitude of evoked excitatory junction potentials (EJP) (Figures 1 and 2). The decrease in size of the EJPs was alwayr much larger than that which might be expected from the degree of depolarization. The reduction of the EJPs was seen in sensitive preparations with the lowest concentration of PGE_1 (10 ng/ml) and became more pronounced when the concentration was increased. In most preparations maximal inhibition was obtained at about 100 ng/ml of PGE_1 and a further increase in concentration of the drug added very little to the inhibition already obtained. As with the depolarizing action of PGE_1, there were rather large

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differences in sensitivity among the preparations to this inhibitory effect on the EJPs. Moreover, the inhibition of the EJPs could be pronounced in a preparation which showed only slight depolarization and vice versa. When the inhibition was moderate, facilitation of the EJPs still occurred in response to successive stimuli and to increases in stimulation frequency or voltage (Figure 1). In sensitive preparations, however, single EJPs in response to low frequency stimulation (0.1–1 imp/sec) could be almost totally abolished (Figure 2f) even when they were evoked at maximal voltage. Summation of EJPs in response to stimulation with frequencies larger than 1 imp/sec still occurred even when the individual EJPs in response to low frequency stimulation were almost invisible (Figure 2g). Because of the inhibition by PGE_1 of the EJPs, individual or summating EJPs in the lower range of stimulation frequencies failed to reach the threshold for anitiation of an AP (Figure 2c). However, with higher stimulation frequencies it was always possible to obtain APs provided that the stimulation voltage was maximal. The exact frequency at which threshold was reached after PGE_1 administration varied from preparation to preparation depending on the degree of inhibition of the single EJPs and the degree of depolarization of the smooth muscle. In general it was not possible to obtain APs with frequencies lower than 3 imp/sec, and always possible to obtain APs with frequencies larger than 15 imp/sec in the presence of PGE_1 (100 ng/ml). After removal of PGE_1 complete recovery of the EJPs was generally obtained within 15 min.

When PGE_1 had produced a distinct depolarization of the smooth muscle, there was usually a change in the configuration of the propagated APs once they were obtained. They generally became larger and had also sometimes a shorter duration. Further, in preparations having a tendency to 'split' or even 'double' spikes (Figure 2 b), the pattern in general changed to on single large spike (Figure 2d). Concomitantly the contraction of the organ became larger (Figure 2d).

Atropine 1 μg/ml did not counteract any of the actions of PGE_1.

Discussion and conclusions. HEDQVIST and BRUNDIN[6] have shown that PGE_1 can inhibit adrenergic transmitter release in the cat spleen. This observation has been confirmed on the rabbit heart (cf. ref.[7,8]). It is also probable that PGE_1 depresses cholinergic transmission in the latter preparation[9]. It is not likely that prostaglandins have a postjunctional inhibitory action on the transmitter in the guinea-pig vas deferens[10]. Therefore the decrease in size of the EJPs seen after PGE_1 appears to provide electrophysiological support for the assumption that prostaglandins can depress autonomic neurotransmitter release. The inhibitory effect of PGE_1 on the motor response of the vas deferens to nerve stimulation can therefore be ascribed to the depression of the EJPs.

The potentiation seen on other occasions is probably due to the direct depolarizing effect of the drug on the postjunctional membrane of the smooth muscle cells of the organ, bringing the membrane potential closer to the firing level and promoting propagation of APs in the tissue. An effect on calcium influx to the cells and hence a promotion of the contractile processes can, of course, not be excluded. This effect of PGE_1 is probably similar to that of acetylcholine and other smooth muscle stimulants on the vas deferens preparation[11].

It is tempting to suggest that the inhibitory effect of PGE_1 on the EJPs and its smooth muscle depolarizing action have a common basis. If the main effect of prostaglandins consists of opening ion channels in membranes, prostaglandins might depolarize autonomic nerve terminals and act in a similar way as has been proposed for presynaptic inhibition in the central nervous system[12,13].

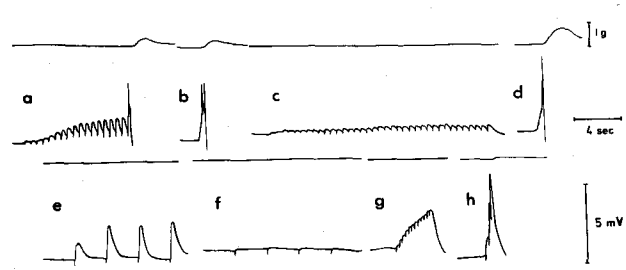

Fig. 2. Facilitated and summating EJPs initiating spikes and contractions. Upper tracing mechanical responses, lower electrical responses. a) 25 V, 2 imp/sec. b) 15 V, 10 imp/sec. c) Same as a) in the presence of PGE_1 100 ng/ml. d) Same as b) in the presence of PGE_1 100 ng/ml. e–h) From another preparation. e) 30 V, 0.4 imp/sec. f) Same as e) in the presence of PGE_1 100 ng/ml. g) Stimulation frequency increased to 4 imp/sec. h) 4 imp/sec 20 min after PGE_1. for further explanation see text.

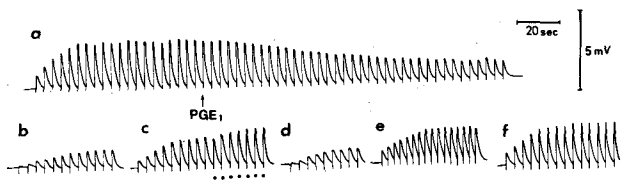

Fig. 1. EJPs in response to transluminal nerve stimulation. a) 20 V 0.3 imp/sec. At arrow PGE_1 20 ng/ml. b) Facilitated EJPs in the presence of PGE_1 20 ng/ml same stimulation parameters as in a. c) Voltage increased to 30 V and at dots to 40 V. PGE_1 20 ng/ml. d) Control period same conditions as in b). e) Frequency increased to 0.4/sec other parameters as in b) and d). f) 15 min after PGE_1 same stimulation parameters as in a), b) and d). For further explanation see text.

Résumé. La prostaglandine E_1 diminue les potentiels de jonction excitateurs du muscle lisse du vas deferens de cobaye en réponse à une stimulation nerveuse, mais provoque aussi une dépolarisation modérée de ce tissu. L'effet inhibiteur de la drogue sur la réponse motrice de cet organe à une stimulation nerveuse est attribué à la 1e de ces actions. L'effet stimulant sur la réponse motrice parfois constaté, est attribué à la seconde action.

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