inhibition was shorter (53 min vs 74 min) with the other characteristics being similar (Table 1).

Various analogs of somatostatin were examined under similar conditions at 0.6 μmole/kg, s.c. Di-Gly-S and Leu-di-Gly-S exhibited activities, with respect to the duration and degree of inhibition, similar to somatostatin (86 min; 71%) (Table 1). Leu-di-Gly-[descarboxy^{14}]-S was shorter in duration of action (41 min) while being as effective in degree of inhibition. [Des-Ala^{1}Gly^2][desamino^3][descarboxy^{14}]-S was shorter in duration of action than somatostatin (39 min vs 90 min) but exhibited a similar degree of inhibition (60% vs 59%); similar activities were exhibited by [Des-Ala^{1}Gly^2][acetyl^3][descarboxy^{14}]-S (43 min vs 74 min; 72% vs 75%). Each of the analogs was similar to somatostatin with respect to the maximal inhibitory effect and time in reaching this effect with the difference being that the less active analog exhibited a more rapid rate of return to control secretory levels than somatostatin (Fig. B, C). The less active analogs exhibited about one-half the duration of action as somatostatin.

Somatostatin at 1×10^{-7} *M* inhibited by about 50% the adenohypophyseal cyclic AMP accumulation induced by PGE_2 (Table 2). At the lower concentration of 0.2×10^{-7} *M*, somatostatin did not cause a significant inhibition. Similar inhibitory activities at these concentrations were exhibited by the di-Gly-, tri-Gly- and [Des-Ala^{1}Gly^2][acetyl^3][descarboxy^{14}]-S and [Des-Ala^{1}Gly^2][desamino^3][descarboxy^{14}]-S analogs. The retro-enantio-derivative of the latter analog was ineffective even at 1×10^{-4} *M* as was [desamino^1][descarboxy^{14}]-retro-enantio-S; the latter analog exhibited a significant decrease at 1×10^{-3} *M*.

Discussion. The findings show that for inhibition of basal gastric acid secretion elongation of somatostatin at the amine terminal by a di-Gly or Leu-di-Gly does not prolong the action. The terminal carboxyl group in the elongated analog, i.e., Leu-di-Gly-S, is of importance for duration of action since the analog lacking this group exhibited a shorter duration of activity. This latter observation is consistent with the findings under similar conditions that the somatostatin analog lacking the terminal carboxyl group and also the somatostatin analog lacking the terminal carboxyl and amino groups were also shorter in duration of action than somatostatin[2]. The ring portion of somatostatin, i.e., the ring peptide chain containing the 2 linked cysteine moieties, is sufficient for maximal inhibitory activity, but not for the duration of action, as this analog exhibited a shorter duration of action; the duration of action is not enhanced to that of somatostatin by the presence of an acetyl group on the amine terminal.

The results obtained indicate that for inhibition of cyclic AMP accumulation elongation of somatostatin at the amine terminal by a di-Gly or tri-Gly does not decrease the inhibitory activity; further, the activity is not increased under the conditions examined. In similar activity studies the terminal amino group was also shown to be unessential for the inhibitory activity[8]. The ring portion of somatostatin is sufficient for maximal activity. The nature of the amine terminal in the latter type of analog does not appear to be of importance as the presence of an acetyl group does not alter the activity; this is also consistent with the above observations. In the ring portion of somatostatin reversal of the direction of the peptide bond and inversion of all asymmetric centers, i.e., the retroenantio isomer of the ring portion, causes loss of the activity even at much higher concentrations. In accord are the findings that the analog lacking the terminal amino and carboxyl groups is effective[8] whereas the retroenantio isomer is relatively ineffective (present studies).

With the somatostatin analogs examined in the present and previous studies[2,8], it appears that the pituitary and gastrointestinal receptors exhibit similar recognition specificity. Differences in the gastrointestinal activities of the analogs appears to reflect differences in the duration of availability of the analogs rather than receptor affinity.

Pimozide and pyrogen-induced fever in rabbits

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Summary. Pimozide, a selective blocker of DA receptors, partly inhibits but does not suppress either the DA-induced hyperthermia or the LPS-induced fever in rabbits. This suggests that a common DA-related mechanism could be at least partly involved in both responses.

Pimozide, a selective blocker of dopamine (DA) receptors[3] has been reputed to inhibit the hyperthermic effects of apomorphine, amphetamine and L-dopa in rabbits[4-6], and for this reason it was suggested that dopamine was involved in these effects. Since there is no data on the possible interference of pimozide with bacterial pyrogen-induced fever, we investigated the effects of this substance when administered to rabbits by different routes alone and before the injection of either DA or E. coli lipopolysaccharide (LPS).

Materials and methods. The experiments were performed on male mongrel rabbits, with a mean body weight of 2.5 kg. They were kept in a temperature-controlled environment (20° ± 1) for a week and during the whole experiment. All the material used were made pyrogen-free by treating at 180 °C for 3 h. Pimozide was dissolved in 100 μl glacial acetic acid and then diluted to 2.5 ml in the pyrogen-free 0.9% NaCl solution (pH = 3.5). The pyrogen solution (E. coli LPS, DIFCO W.E. Coli 0127 B8) and dopamine (HCl) were also made up in pyrogen-free 0.9% NaCl solution. Injections were made either i.p. or into the lateral ventricle (i.c.v.). For cannulation of the lateral ventricle in rabbits, the method was that described by Hasselblatt and Sproull[7]. The sequence of

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2 Acknowledgments. We wish to thank Laboratories Le Brun (Paris) for the generous supply of pimozide.

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injections was as follows: a) 150 µl of 0.9% NaCl as a control, followed by 100 µl of either the solvent, pimozide, DA or LPS followed by an injection of 50 µl 0.9 NaCl; b) 150 µl of 0.9% NaCl followed 2 h later by the same selection of pimozide, DA or LPS. The results reported were obtained from 5 to 8 individual measurements.

Results. Pimozide effects when injected i.p. A convulsive effect similar to that produced by pentetrazol was often observed with 4 mg/kg pimozide i.p., the dose used by Hill and Horita[5]. When this dose was followed by LPS 1.5 µg/kg i.v. 4 h later, most rabbits showed febrile responses. On the other hand, this dose of pimozide only partly prevented the development of hyperthermia in response to DA (400 µg i.c.v. injected) (figure 1a). The statistical analysis of the results showed no significance between curves 1 and 2 when the analysis was performed at each point with the Student's t-test. However, the statistical evaluation of the period from 0.5 to 1.5 h showed a significant difference ($p \leqslant 0.05$). Furthermore, the comparison of the fever index (F.I.) at point 2 h also showed a difference (DA F.I. = 100; Pimozide + DA F.I. = 52). Those differences in statistical analysis were probably due to the limited number of results at each point and the variations in individual responses.

Pimozide effects when injected i.c.v. Intraventricular injection of 100 µl of the solvent caused a slight hyperthermia (0.5 °C ± 0.06 in 0.5 h) which had subsided in 2 h. In consequence of this solvent effect, results with the drugs are only considered after two hours have elapsed. Such a procedure is consistent with the long-lasting effect of pimozide[8].

The pimozide doses were 0.25, 0.5 or 4 µg followed 2 h later by either 400 µg DA or 1.5 ng LPS i.c.v. Convulsive effects occurred only when the dose of pimozide was greater than 4 µg. DA hyperthermia was reduced only by 4 µg pimozide (figure 1b) (DA F.I. = 100; Pimozide + DA F.I. = 27), whereas LPS fever was reduced by both 1 µg and 4 µg doses (figure 2). Statistical evaluation of the period from 2.5 to 4 h showed that the decrease in LPS febrile response with pimozide was highly significant ($p < 0.001$) as compared to the response with LPS control; the effect of the 2 dose levels were not significantly different.

Discussion. The elevation in temperature produced by i.c.v. administration of DA was reduced but not abolished when pimozide was given by the same route. Intraperitoneally injected pimozide had a lessened effect which could explain the inability of pimozide when injected i.p.

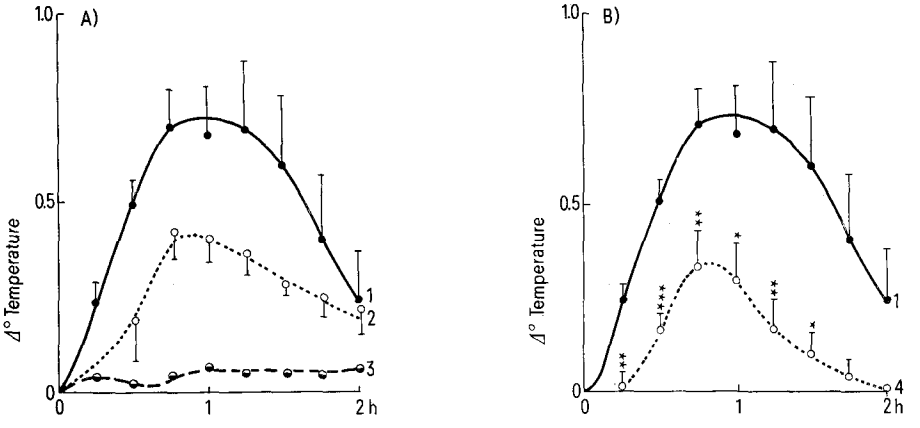

Fig. 1. Effect of pimozide on the time course of dopamine-induced hyperthermia in rabbits. A) Curve 1: DA 400 µg i.c.v. Curve 2: pimozide 4 mg/kg i.p. following 4 h later by DA 400 µg i.c.v. Curve 3: Controls (NaCl 0.9% i. c. v.). Significance between curves 1 and 2: see text. B) Curve 1: DA 400 µg i.c.v. Curve 4: pimozide 4 µg i.c.v. following 2 h later by DA 400 µg i. c. v. Significance between the points of curves 4 and 1: * $p \leqslant 0.05$, ** $p \leqslant 0.01$, *** $p \leqslant 0.001$.

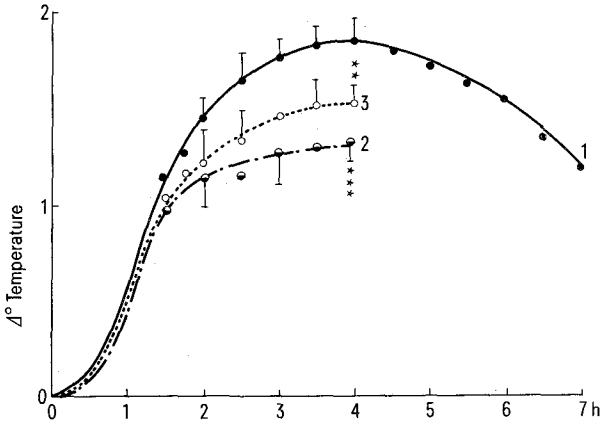

Fig. 2. Inhibition of i.c.v. LPS-induced fever in rabbits treated with pimozide. Curve 1: LPS 1.5 ng, i.c.v. Curve 2: pimozide 4 µg, i.c.v., 2 h before LPS. Curve 3: pimozide 1 µg, i.c.v., 2 h before LPS. Significance against the controls (curve 1): ** $0.02 < p < 0.01$, *** $0.01 < p < 0.001$.

to block hyperthermia induced by large dosis of DA (1 mg/kg^{-1} i.c.v.), as shown by Girault et al.[9]. This suggests that DA hyperthermia may be only in part due to a direct stimulation of brain DA receptors. However, when pimozide was injected i.p. the amount of drug which reached the relevant receptors in an active form might be so small as to be unable to inhibit the DA effect. Since pimozide also partly suppresses LPS fever when injected i.c.v. in rabbits, this is consistent with the hypothesis that DA receptors may be involved to some extent in LPS-induced fever. Such an hypothesis may be relevant to the results of Gardey-Levassort et al.[10], who observed decreased levels of DA metabolites in the brain during the early phase of pyrogen fever in rabbits, as well as previously found by Roos[11] after the injection of apomorphine, a dopamine receptor stimulant.

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