tensive effects are mediated by a direct α-adrenoceptor agonist action on the central nervous system (Bolme & Fuxe, 1971). The effect of pre-treatment with desmethylimipramine (DMI) 20 min before intravenous clonidine (3–100 μg/kg) and intracisternal clonidine (1 and 3 μg/kg) was investigated in groups of normotensive rabbits (n=4–7). Arterial pressure was measured directly with a strain gauge transducer via a catheter in the central artery of the ear.

DMI (2·5 mg/kg, i.v.) caused a transient reduction in mean arterial pressure. Clonidine was given twenty min after DMI. At this time, the arterial pressure was not significantly different in control and DMI pre-treated groups, but the pressor sensitivity to intravenous noradrenaline was increased in DMI treated animals (mean dose ratio 4·3 : 1, n=4). The hypotensive action of intravenous clonidine in unanaesthetized rabbits was markedly reduced at all doses tested. The acute transient pressure rise after clonidine did not differ significantly in the two groups (control 24·8 ± S.E. of mean 3·28 mmHg; DMI pretreated 25·6 ± S.E. of mean 2·68 mmHg). Clonidine 30 μg/kg reduced the mean arterial pressure by 17·6 ± S.E. of mean 3·57 mmHg in controls, and 5·3 ± S.E. of mean 0·99 mmHg in DMI pretreated animals. Clonidine 1 μg/kg injected intracisternally in sodium pentobarbitone anaesthetized rabbits reduced the mean arterial pressure by 37·0 ± S.E. of mean 2·33 mmHg in controls while in DMI pretreated animals, the fall was only 10·2 ± S.E. of mean 2·67 mmHg.

These results indicated that the hypotensive effect of intravenous and intracisternal clonidine is greatly reduced by DMI. It is proposed that clonidine exerts its depressor effect by an action on noradrenergic neurones and not as a direct receptor agonist. This hypothesis is supported by observations that clonidine inhibits the release of noradrenaline following nerve stimulation from the isolated rabbit heart (Werner, Starke & Schümann, 1972) and that destruction of central noradrenergic neurones with 6-hydroxydopamine abolishes the hypotensive effect of centrally administered clonidine (Reid & Dollery, 1972).

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Mode of action of permeability factor obtained from the spleen

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Spleen permeability factor (SPF) is a protein fraction obtained from rat spleen by homogenization of the sliced tissue followed by ammonium sulphate fractionation. When injected intradermally in rats it causes an increase in capillary permeability demonstrable by dye extravasation. Dye leakage was quantitatively

assessed by extraction and colorimetric assay. Samples of SPF contain no appreciable amounts of low molecular permeability factors and produce no contraction or relaxation of isolated smooth muscle.

The permeability effect of SPF is exerted indirectly through the release of histamine and 5-hydroxytryptamine (5-HT) from mast cells as indicated by the following findings (1) the blueing effect of SPF is almost completely abolished by the previous systemic application of a combination of mepyramine and methysergide in doses which specifically antagonize histamine and 5-HT respectively; (2) both histamine and 5-HT are released by SPF from rat peritoneal mast cells *in vitro*; (3) after depleting rat skin of histamine and 5-HT with compound 48/80 in doses which do not impair the permeability effect of histamine, SPF loses its permeability effect. The histamine releasing effect of SPF in isolated mast cells is abolished by 0·1 mM iodoacetate, but in contrast to anaphylactic histamine release it is unaffected by Ca-lack and by raising temperature to 45° C for 5 min.

Chemical modification of spleen permeability factor

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The permeability factor obtained from the spleen (SPF), referred to in the preceding communication, is a mixture of priteins extracted from the spleens of pig or rat. Attempts have been made to purify those components responsible for the permeability activity. Gel filtration, molecular membrane filtration, ion-exchange chromatography, acrylamide electrophoresis, and isoelectric focussing yield a variety of protein fractions from SPF, electrophoretically and chromatographically distinct, which possess biological activity comparable to that of the original material. This activity may be due to structural features shared by many proteins, likely to be of a simple type depending on distribution of amino acid side chains rather than complex features of tertiary structure.

The use of reagents which modify the proteins of SPF has helped to define those features essential for permeability activity. Two categories of reagents have been used; those which unwind the tertiary structure of proteins, and those which react with particular amino acid side chains to effect a chemical modification. In practice these two effects are not clearly separable, since extensive chemical modification will produce changes in tertiary structure, whilst maximum disruption of tertiary structure is effected only by reagents which cause some chemical modification.

The treatment of SPF with modifying agents has led to the following findings:

(1) The unfolding of tertiary structure followed by re-folding, as caused by prolonged exposure to 6M urea and its removal prior to bioassay, does not diminish biological activity.

(2) Further disruption of tertiary structure by similar treatment with urea plus mercaptoethanol reduces activity threefold. Blockage of native and formed sulphydryl groups causes no further reduction in activity.

(3) Blockage of ϵ-amino groups of lysine and α-amino terminal groups with three different reagents causes a reduction in activity to 1/20th of the original level.