Three considerations support the view that inhibition by aspirin and morphine of the constriction response to acetylcholine, bradykinin and potassium is antinociceptive. (1) The response probably arises from stimulation of nociceptors; (2) aspirin and morphine are known to have analgesic action in man; (3) aspirin does not show other forms of anticholinergic action, such as antagonism of physostigmine toxicity. The differences in potency of aspirin against constriction induced by acetylcholine, bradykinin and potassium suggest that it is acting peripherally on different chemonociceptors, in contrast to morphine which is equally effective against all three noxious agents and is believed to act centrally. These views are consistent with the conclusion of Lim, Guzman, Goto, Braun and Rodgers[13], from cross-circulation experiments, that in dogs aspirin acts peripherally and morphine centrally against nociception induced by bradykinin. In anti-inflammatory tests in mouse peritonitis, aspirin is little more potent than sodium salicylate[7,14]. Its different potency from salicylate against acetylcholine-induced constriction, together with the small latency of the response inhibited, suggest that aspirin may exert its antinociceptive action directly, rather than by reducing fluid exudation from blood vessels. The higher potency of aspirin than sodium salicylate implies that this action does not depend on conversion of aspirin to salicylate.

An alternative, but less likely, explanation of the different effectiveness of aspirin against abdominal constriction in mice induced by acetylcholine, bradykinin and potassium is that aspirin distinguishes in the central nervous system between nociception induced by these three substances.

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Inhibition of Uptake of Tritiated-noradrenaline in the Intact Rat Brain by Imipramine and Structurally Related Compounds

Imipramine is one of the most effective drugs for the alleviation of mental depression. There is little information concerning the mechanism of action of this drug at the biochemical level. An impressive body of evidence has been accumulating over the past few years indicating that noradrenaline is involved in behaviour. Although other antidepressant drugs such as monoamine oxidase inhibitors[1], and amphetamine[2], affect cerebral noradrenaline concentration, imipramine was found to have no measurable effect on the level of the neurohumour in the brain[3]. It has been shown that imipramine blocks the uptake of noradrenaline in peripheral tissues[4] and brain slices[5]; however, many other drugs including the tranquillizer chlorpromazine have the same action[5,6]. Because noradrenaline cannot cross the blood-brain barrier, it was virtually impossible to examine directly the action of imipramine or other drugs on the uptake of noradrenaline by the intact brain. We have recently described a procedure which made it possible to introduce tritiated-noradrenaline of high specific activity into the rat brain by an intraventricular injection. The tritiated-noradrenaline is taken up and retained by nerve endings in the brain and then shows the essentially same biochemical behaviour as the endogenous neurohumour[7]. This technique enabled us to investigate the effect of imipramine on the uptake of tritiated-noradrenaline by the intact rat brain. It will be shown that imipramine and structurally related antidepressant drugs but not chlorpromazine block the uptake of tritiated-noradrenaline in the brain.

Sprague-Dawley male rats weighing 300 g received imipramine 1 h before the injection of tritiated-noradrenaline (0·07 μg) into the lateral ventricle of the brain. Two hours after the intraventricular administration of the catecholamine the rats were killed and the brain assayed for tritiated-noradrenaline[8].

Table 1. Inhibition of Tritiated-noradrenaline Uptake in the Rat Brain by Antidepressant Drugs

Treatment	Amount given (mg/kg)	Clinical antidepressant action‖	% of control value
Imipramine	20	Yes	63 ± 3·1*
Desmethylimipramine	20	Yes	64 ± 3·2*
Amitriptyline	10	Yes	77 ± 7·6†
Compound II‡	20	No	99 ± 4·2
Compound III§	20	No	93 ± 3·5
Chlorpromazine	20	No	107 ± 8·6
Chlorpromazine	40	No	91 ± 6·7

* $P < 0.001$. † $P < 0.05$.

Groups of six rats were given the drugs intraperitoneally 1 h before the intraventricular administration of 0·07 μg of *d,l* 7-³H-noradrenaline (5·8 c./m.mole from New England Nuclear Corporation, Boston). Rats were killed 2 h later and the brain assayed for tritiated-noradrenaline. Brain from untreated rats had about 30,000 c.p.m. per g of tissue. Results are expressed in percentage of the control brain tritiated-noradrenaline ± *S.E.M.*

‡ Compound II has the same structure as imipramine except that a dimethylaminoisopropyl side chain is substituted for a dimethylaminopropyl side chain.

§ Compound III has the same structure as chlorpromazine except that a dimethylaminoethyl, ethyl ether side chain is substituted for a dimethylaminopropyl side chain.

‖ Information concerning the antidepressant activity of the various compounds was kindly provided by Dr. Murray Weiner, Geigy Research Laboratories, Yonkers, N.Y.

The uptake of tritiated-noradrenaline by the brain of imipramine-treated animals was markedly reduced (Table 1). The effect of congeners of imipramine and other compounds related in structure, such as amitriptyline and the tranquillizer chlorpromazine, on the uptake of tritiated-noradrenaline was also examined. Desmethylimipramine and amitriptyline reduced the uptake of tritiated-noradrenaline; both these drugs have been found clinically active as antidepressants. A closely related derivative of imipramine with a dimethylaminoisopropyl side chain instead of the dimethylaminopropyl side chain which was found to be clinically inactive as antidepressant had no effect on the uptake of tritiated-noradrenaline in the brain. Chlorpromazine, a tranquillizing drug, which can block the uptake of tritiated-noradrenaline in peripheral tissues and brain slices, had no effect in the intact rat brain.

The uptake of noradrenaline into storage granules of peripheral nerve endings is a major mechanism for the inactivation of noradrenaline[8]. In the periphery it has been shown that drugs which block the uptake of noradrenaline enhanced the physiological actions of the neurohumour[9]. Noradrenaline has been shown to be liberated from nerve endings, react with adrenergic recep-

tors, and then return in part to the stores in the sympathetic nerves[10]. It is likely that a similar mechanism of noradrenaline release and re-uptake is operating in the brain. The experiments described here show that imipramine, desmethylimipramine and amitryptyline block the uptake and binding of noradrenaline by the brain. On the other hand, these structurally related compounds which are devoid of clinical antidepressant activity have no effect on the uptake of tritiated-noradrenaline in the brain. The ability of imipramine to prevent the rebinding of noradrenaline by cerebral tissues may be a mechanism for the antidepressant action of this drug. Such an action of the drug would allow more free physiologically active noradrenaline liberated from the central sympathetic neurones to react with the central adrenergic receptors. The observations that imipramine potentiates the peripheral action of administered noradrenaline[11], that desmethylimipramine will cause hyperactivity after a rapid release of noradrenaline produced by reserpine[12], and that both will potentiate the stimulating action of amphetamine[12,13], a drug that releases noradrenaline in the brain[2], support the mechanism of action of the antidepressant drugs proposed in this communication.

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IMMUNOLOGY

Breaking of Immunological Paralysis by Injection of a Specific Depolymerase

THE elaboration by the soil organism *Bacillus palustris* of a depolymerase (*D*3) specific for the long-chained capsular polysaccharide of type III *Diplococcus pneumoniae* (*S*III) (ref. 1) provides an opportunity to explore some of the mechanisms of immunological paralysis induced by *S*III. The polymerase when incubated *in vitro* for prolonged periods with *S*III cleaves it to hexasaccharides and smaller units[2]. It could readily be shown that when the products of this reaction are injected into adult mice they do not elicit an immune response. Thus, 5/5 mice injected with 0·5 ml. of a 21-h reaction mixture containing 40 μg *S*III and 50 viscosity units of *D*3 failed to form antibodies. When these mice were later injected with 0·1 μg *S*III they all formed antibodies against the *S*III. When *D*3 is injected intravenously into mice which 12 h previously had received 100 μg *S*III it rapidly clears the antigen from the circulation so that within 12 h *S*III is no longer detectable by either the micro-precipitin or the complement fixation tests. The former test detects 0·1 μg and the latter 0·01 μg antigen. Although no *a priori* evidence existed it seemed just possible that the enzyme might even enter the cells containing the *S*III and relieve the immunological paralysis caused by the injection of relatively large amounts of *S*III.

Table 1. EFFECT OF TYPE III DEPOLYMERASE ON IMMUNOLOGICAL PARALYSIS

*S*III (μg)	Interval between *S*III and *D*3 (days)	Bled after *D*3 (days)	Titres*	No. mice released from paralysis on basis of h.a. challenge†	
0·1	—	7	32 64 64 64		
0·1	2	7	8 64 64 32		
100	1/4	7	8 16 8 8	4/4	2/4
100	1	7	8 16 16 8 4 0 8 0	6/8	5/8
100	2	6	4 8 0 32 8 16 4 8 4 4 4 8	11/12	3/4
100	3	6	16 16 16 8 16 8 8 8 8 4 8 8 4	13/13	4/8
100	4	5	0 4 0 8 32 16 32 16	6/8	5/8
100	5	5	0 0 0 0	0/4	2/4
100	6	7	8 0 0 0	1/4	0/4
100	7	7	8 4, 7×0	2/9	
100	8	5	0 0 0 0	0/4	2/4
100	18	7	4 4 4 4, *14*×0	4/18	3/14
100	69	7	*10*×0	0·/10	
100	92	7	*13*×0	0/13	
2,000	1	7	9, 32 16	3/3	
2,000	2	7	16 16 16 8 8 4	6/6	
2,000	3	7	4 16 4 4 4 4	5/5	
2,000	4	7	8 8 8 0	3/4	
2,000	5	7	4 0 4 4	3/4	
2,000	6	7	8 4 4 4	4/4	
2,000	7	7	4, 4, 16, 4, 0, 0 0	4/7	
2 000	10	7	4 0 4 0	2/4	
2,000	69	7	*14*×0	0/14	

* Titrations were commenced with a serum dilution of 1 in 4. The figures are the reciprocals of the serum dilution at which an end-point was noted.
† Live/total. Not all mice were challenged.

Four separate experiments were done over 2 years, but for convenience the results are presented collectively. Two measures of immunity were used. Mice were bled from the retro-orbital sinus and their sera, after absorption with sheep cells, titrated for antibodies to *S*III using as antigen a filtrate of type III *D. pneumoniae* coated to sheep erythrocytes[3]. In some experiments some mice were challenged about one month after *D*3-treatment with 100–200 lethal doses of type III *D. pneumoniae*.

The intraperitoneal injection of 0·1 μg *S*III into 12–14-week-old male *CAF* mice results, whether or not it is followed 2 days later by the intravenous injection of *D*3, in the formation of relatively high titres of antibodies to *S*III (Table 1). On the other hand, when mice were injected with 100 μg or 2·0 mg *S*III and bled at intervals over the next 24 weeks *S*III antibodies were never detected. Entirely different results were noted when *D*3 was injected into the paralysed mice. If the enzyme was injected 6 h to 4 days after the paralysing dose of *S*III, then the mice were no longer paralysed and indeed 40/45 animals formed detectable—albeit relatively low-titred—antibodies against *S*III. As the interval between the injection of antigen and enzyme was increased fewer mice were relieved of their paralysis and they formed only low titres of antibodies against *S*III. Thus, only 7/39 paralysed mice injected with *D*3 5–18 days after *S*III and 0/23 injected 2–3 months after *S*III formed detectable antibodies. The results of the challenge test essentially confirm these findings and the slightly less impressive results obtained with this method (the figures are 19/32 for the period 6 h–4 days; 7/26 for the period 5–18 days and 0/5 for the 2–3-month period) might be attributed to the waning of immunity in the interval between the injection of *D*3 and the subsequent challenge. As an additional control some of the paralysed mice were injected intravenously with saline at the same time as other mice were injected with *D*3. They were still paralysed when bled one week later.

Similar results were obtained with those mice injected with 2·0 mg *S*III. Seventeen of 18 mice injected with *D*3 during the 4 days after the *S*III injection were freed from their paralysis: 13/19 injected with *D*3 between 5 and 10 days after *S*III were also freed of paralysis whereas 0/14 injected with *D*3 69 days after *S*III were able to produce detectable antibodies to *S*III.