genate while more than 13% was present in liver. The small proportion of Δ^1-THC-7-oic acid reaching the brain suggests the possibility that it may exert most of its inhibitory action at sites not in the central nervous system. If this is the case, it follows that the major primary site of cataleptic action of Δ^1-THC could also be located outside of the brain. This is supported by the data in table 2 showing that the i.p. injection of PGE_2 and PGI_2 gave a THC-like response in the ring test.

The finding reported here that a major metabolite of Δ^1-THC has in vivo biological activity that antagonizes the effects of the parent drug should be considered in any studies with THC where metabolism can occur. For example, pharmacokinetic studies have yielded data which suggest that the plasma levels of THC do not correlate well with the intensity of its effects[13]. Such a self-generated antagonism as we have described for THC could provide a basis for a lack of correlation except in the special situation where THC and its antagonist are always present in the same ratio at the site of action.

In terms of mechanism of action of THC, this report extends our previous findings based on an in vitro model[7]. A role for prostaglandins in the pharmacodynamics of THC, thus, seems to be a reasonable hypothesis. It is interesting to note that throughout its long history cannabis has been considered to have anti-inflammatory properties[14]. These reports can now be better explained on the basis of the cycloxygenase inhibitory action of Δ^1-THC-7-oic acid[6] rather than Δ^1-THC itself.

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2 Burstein, S., Biotransformations of the Cannabinoids, in: Pharmacokinetics and Pharmacodynamics of Psychoactive Drugs. Eds G. Barnett and C.N. Chiang. Biomed Publ., Forest City, California 1985.
3 Burstein, S., Hunter, S.A., Ozman, K., and Renzulli, L., *In vitro* models of cannabinoid-induced psychoactivity, in: 'Marihuana '84'. Ed. D.J. Harvey. IRL Press, Oxford 1985.
4 Burstein, S., Hunter, S.A., Ozman, K., and Renzulli, L., Biochem. Pharmac. *33* (1984) 2653.
5 Burstein, S., Hunter, S.A., and Renzulli, L., J. Pharmac. exp. Ther. *235* (1985) 87.
6 Burstein, S., Hunter, S.A., Latham, V., Mechoulam, R., Melchior, D.L., Renzulli, L., and Tefft, R.E. Jr, Life Sci. *39* (1986) 1813.
7 Burstein, S., Hunter, S.A., Latham, V., and Renzulli, L., Biochem. Pharmac. *35* (1986) 2553.
8 Pertwee, R.G., Br. J. Pharmac. *46* (1972) 753.
9 Ohlsson, A., Widman, M., Carlsson, S., Ryman, T., and Strid, C., Acta pharmac. toxic. *47* (1980) 308.
10 Fairbairn, J.W., and Pickens, J.T., Br. J. Pharmac. *67* (1979) 379.
11 Perez-Reyes, M., Pharmacodynamics of Certain Drugs of Abuse, see ref. 2.
12 Wall, M.E., and Brine, D.R., in: Marihuana: Biological Effects, pp. 36–38. Eds G.G. Nahas and W.D.M. Paton. Pergamon, Oxford 1979.
13 Hollister, L.E., et al., J. clin. Pharmac. *21* (1981) 171S; Cocchetto, D.M., Owens, S.M., Perez-Reyes, M., DiGuiseppi, S., and Miller, L.L., Psychopharmacology *75* (1981) 158.
14 Sophia, R.D., Nalepa, S.D., Harakal, J.J., and Vassar, H.B., J. Pharmc. exp. Ther. *186* (1973) 646.

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Development of high blood pressure in spontaneously hypertensive rats is delayed by treatment with cyclosporin at an early age

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Summary. In spontaneously hypertensive rats the effect of the T-cell inhibitor cyclosporin was studied at different ages. If treatment was started at the age of 2 weeks the development of hypertension was delayed, but the ultimate level of blood pressure was not affected. These results indicate the involvement of immune mechanisms in the early development of hypertension in spontaneously hypertensive rats.

Key words. SHR; cyclosporin; immune mechanisms; hypertension.

In recent years immunological abnormalities have been reported to be present in a strain of spontaneously hypertensive rats (SHR) originally developed by Okamoto and Aoki[1] by selective inbreeding of Wistar Kyoto rats (WKY).

Cell-mediated immune responses, including mitogenic responses, are reduced in SHR. This suggests a selective suppression of T-lymphocyte function in SHR, the cause of which may be a natural cytotoxic autoantibody against T cells[2–5]. This autoantibody occurs from the age of about one month onward, its titer varying widely among individuals[4,5].

The role of the immunological depression in the development of hypertension in SHR is as yet unclear, but restoration of the immune responsiveness by transplantation of histocompatible thymus tissues into neonatal SHR suppressed the development of hypertension[6]. Administration of thymosin (fraction 5) to young and adult SHR resulted in restoration of T cell functions and lowering of blood pressure[7]. Since anti-rat-thymocyte serum and implantation of WKY thymuses also proved capable of reducing blood pressure in SHR, it is possible that hypertension in SHR has an immunological basis[8–10]. Results of cyclophosphamide treatment of SHR are as yet inconclusive, since the observed decrease in blood pressure was accompanied by a reduced growth rate[11,12].

We report the effect of treatment with cyclosporin on the development of hypertension in SHR. Cyclosporin is a nonpolar cyclic oligopeptide immunosuppressive agent of fungal origin, which appears to act specifically at the level of the T helper cell and reversibly inhibits the production of lymphokines[13–15].

Materials and methods. Male rats of strains SHR and WKY were obtained from Central Breeding Laboratories TNO, Zeist, The Netherlands. Adult animals (12 weeks old) weighed 180–200 g. Young animals (4 weeks) were used after weaning, weighing 50–60 g, and before weaning (2 weeks old), weighing 20–25 g. Systolic blood pressure was assessed in trained conscious rats by the indirect method of tail sphygmography[16].

Cyclosporin was administered by intragastric gavage, dissolved in a mixture of 96% ethanol, Tween 80 and normal tap water[17,18]. The animals received a dose of 10 mg/kg/day, controls

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were treated in the same way with the vehicle only. Dose adjustments for increase of body weight were made daily. During experiments animals had free access to standard rat food and normal tap water.

Adult animals. After being trained for blood pressure measurement, adult (12 weeks old) SHR received cyclosporin or the vehicle for ten days. Systolic blood pressure and body weight were determined daily.

Young animals. Experiment a. Young SHR were weaned at the age of 28 days and treated with either cyclosporin or the vehicle from that day on for two weeks. On the same day, training for blood pressure measurement was started. Systolic blood pressure values were obtained on the 9th and following days of treatment. Body weight was determined daily.

Experiment b. Cyclosporin or vehicle treatment was started at the age of 14 days. Subsequently , at the age of 28 days, the animals were weaned and training for blood pressure measurement was started. Systolic blood pressure values were obtained from the 11th day of training on. Body weight was determined daily. At the age of 9 weeks, i.e. after 7 weeks of treatment, treatment was discontinued, while body weight and systolic blood pressure were monitored for one more week.

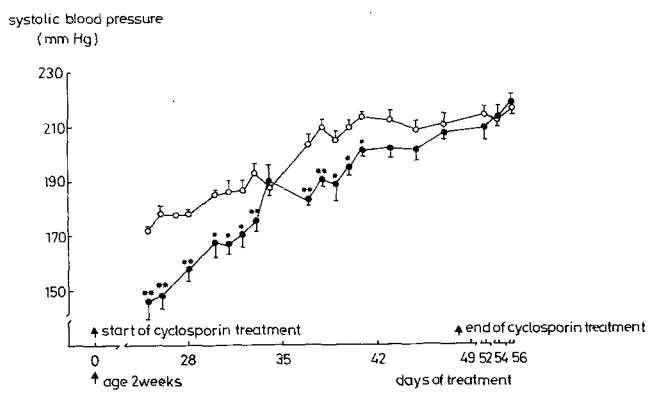
Experiment c. In this experiment exactly the same procedure was followed as described for experiment b. However, instead of SHR, normotensive WKY control rats were used.

Data are presented as means ± SEM. For statistical analysis Student's t-test was employed.

Results. Adult animals. Adult SHR of 12 weeks of age (n = 4–5) were treated with cyclosporin for ten days. The condition of the animals was not affected by the treatment as shown by a normal body weight gain (21 ± 3 g as compared with 22 ± 4 g in controls). Neither systolic blood pressure nor heart rate was affected by the cyclosporin treatment.

Young animals. The results of the experiments a, b and c are summarized in the table. When treatment of SHR was begun at the age of 4 weeks no effect on the development of hypertension or heart rate was observed. If cyclosporin treatment was started at the age of two weeks, systolic blood pressure as measured from the 25th day of treatment onward was significantly lower in cyclosporin treated SHR than in controls (fig.). Blood pressure increased in both groups but more so in the cyclosporin treated animals, so that the difference in blood pressure gradually disappeared. Heart rates were similar in both groups. In 2-week-old WKY cyclosporin treatment did not affect systolic blood pressure, heart rate or growth rate.

Discussion. The present studies indicate that treatment of adult and 4-week-old SHR with cyclosporin does not affect blood pressure or heart rate. In the adult SHR this may be due to the fact that the activity of T suppressor cells is completely inhibited



Effect of cyclosporin treatment on systolic blood pressure of SHR. Data are means ± SEM of 6 rats per group. Administration of cyclosporin (●—●) and vehicle (○—○) was started at the age of two weeks. For details see materials and methods. *p < 0.05; **p < 0.01.

Effect of cyclosporin treatment (10 mg/kg b.wt, once daily p.o.) on body weight, systolic blood pressure and heart rate of young spontaneously hypertensive rats and Wistar Kyoto controls

		Body weight (g)	Systolic blood pressure (mmHg)	Heart rate (bpm)
Experiment a[1]				
	Controls	115 ± 5	188 ± 2	470 ± 14
SHR <				
	Treated	81 ± 5*	185 ± 3	483 ± 5
Experiment b[2]				
	Controls	250 ± 4	210 ± 4	415 ± 10
SHR <				
	Treated	241 ± 9	207 ± 4	432 ± 10
Experiment c[2]				
	Controls	210 ± 7	127 ± 1	385 ± 4
WKY <				
	Treated	189 ± 13	126 ± 2	370 ± 12

Data are means ± SEM of 4–7 rats per group. [1] Results presented were measured at the end of the 2-week treatment period at the age of 6 weeks. [2] Results presented were measured at the end of the 7-week treatment period at the age of 9 weeks. * $p < 0.01$.

by the thymocytotoxic autoantibodies[4,5] and the ensuing immune depression is not changed by treatment with cyclosporin, which inhibits T helper cells[13–15]. Probably, the same argument holds true when cyclosporin treatment is begun at the age of four weeks. Also at this age the autoantibodies are already present[4,5]. Interestingly, it appears that when SHR receive treatment which cyclosporin from the age of two weeks onwards, the development of hypertension in these rats is delayed (fig.). This suggests a modest role for T helper cells in the etiology of high blood pressure in SHR. At the age of two weeks in SHR the thymocytotoxic autoantibodies are not yet present. So far, it is not clear whether cyclosporin treatment in the 2-week-old animals has any influence on the occurrence of the autoantibodies.

The control experiment, in which 2-week-old normotensive WKY rats were treated with cyclosporin, indicated that the delay in the development of high blood pressure is not caused by an action of cyclosporin on blood pressure in general.

Although the body weight in one group of cyclosporin treated SHR (table, experiment a) at the end of the experimental period was lower than in the control group, the increase in body weight and therefore growth was almost normal. The mean body weight of this group of animals was also lower at the beginning of the experiment, albeit not statistically significantly. This is supported by the observation of a normal increase in body weight in SHR treated with cyclosporin from the age of 14 days on (table, experiment b).

Involvement of the immune system in hypertension has been shown in several other animal models. Hypertension induced in rats by renal infarction could be transferred by administration of whole viable lymph node cells to normal rats[19]. Hypertension following allogeneic renal transplantation in rats was effectively prevented by cyclosporin treatment[20]. Furthermore, in human essential hypertension immunological processes may be involved, although it is not clear whether this involvement is primary or secondary[21]. In addition, essential hypertension in humans has been reported to have a genetic basis with a significant incidence in patients possessing HLA B8[22,23].

In conclusion, the present study supports the notion that very early in the development of SHR immunological mechanisms may be involved in the events which result in an increased blood pressure in this strain.

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1 Okamoto, K., and Aoki, K., Jap. Circ. J. *27* (1963) 282.
2 Takeichi, N., and Boone, Ch. W., Cell. Immun. *27* (1976) 52.
3 Takeichi, N., Suzuki, K., Okayasu, T., and Kobayashi, H., Clin. expl Immun. *40* (1980) 120.
4 Takeichi, N., Ba, D.,and Kobayashi, H., Cell. Immun. *60* (1981) 181.
5 Takeichi, N., Suzuki, K., and Kobayashi, H., Eur. J. Immun. *11* (1981) 483.
6 Ba, D., Takeichi, N., Kodama, T., and Kobayashi, H., J. Immun. *128* (1982) 1211.
7 Strausser, H. R., Thymus *5* (1983) 19.
8 Bendich, A., Belisle, E. H., and Strausser, H. R., Biochem. biophys. Res. Commun. *99* (1981) 600.
9 Norman, R. A., Dzielak, D. J., Bost, K. L., Cuchens, M. A., and Khraibi, A. A., Physiologist *26* (1983) A-48 (Abstr. 26.9).
10 Norman, R. A., Dzielak, D. J., Bost, K. L., Khraibi, A. A., and Galloway, P. G., J. Hypertension *3* (1985) 261.
11 Khraibi, A. A., Norman, R. A., and Dzielak, D. J., Physiologist *25* (1982) 337 (Abstr. 70.7).
12 Khraibi, A. A., Norman, R. A., and Dzielak, D. J., Am. J. Physiol. *247* (1984) H722.
13 Borel, J. F., Rüegger, A., and Stähelin, H., Experientia *32* (1976) 777.
14 Britton, S., and Palacios, R., Immun. Rev. *65* (1982) 5.
15 Wagner, H., Trans. Proc. *15* (1983) 523.
16 Leenen, F. H., and Jong, W. de, J. appl. Physiol. *31* (1971) 142.
17 Borel, J. F., Feurer, C., Grubler, H. V., and Stähelin, H., Agent Action *6* (1976) 468.
18 Borel, J. F., Feurer, C., and Magnée, C., Immunology *32* (1977) 1017.
19 Okuda, T., and Grollman, A., Tex. Rep. Biol. Med. *25* (1967) 257.
20 Provoost, A. P., and Keyzer, M. H. de, in: Handbook of Hypertension, vol. 4: Experimental and genetic models of hypertension, p. 518. Ed. W. de Jong. Elsevier Science Publishers, Amsterdam 1985.
21 Kristensen, B. Ø., J. Hypertension *2* (1978) 571.
22 Gualde, N., Michel, J. P., and Safar, M. E., Lancet *2* (1978) 897.
23 Löw, B., Schersten, B., Sanetor, G., Thulin, T., and Mitelman, F., Lancet *2* (1975) 695.

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Differential sensitivity of amphibian nodal and paranodal K^+ channels to 4-aminopyridine and TEA

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Summary. Voltage-dependent K^+ channels are blocked by several drugs, including 4-aminopyridine (4-AP) and tetraethylammonium (TEA). 4-AP is most widely used to localize K^+ channels in mammalian and non-mammalian nerve fibers, but 4-AP and TEA alter various K^+ channels and/or preparations in specific ways. The reason is not known, in part because dissociation constants for 4-AP and TEA have not been measured for nodal and internodal K^+ channels in the same fibers. Smith and Schauf[1] showed that the density of nodal versus paranodal K^+ channels in frog nerves depends on fiber diameter. This size dependence was used to determine the relative sensitivity of nodal and internodal K^+ channels to 4-AP and TEA, and to compare voltage- and time-dependent activation. The results show nodal and internodal K^+ channels activate similarly. However, internodal channels are selectivity blocked by 4-AP while TEA is more effective on nodal channels. A high sensitivity of internodal K^+ channels may explain why 4-AP improves symptoms in diseases such as multiple sclerosis.
Key words. Potassium channels; 4-aminopyridine; tetraethylammonium; *Rana pipiens* nerves; voltage clamp; lysolecithin; nodal channels; internodal channels.

The drug 4-aminopyridine (4-AP) blocks the classical delayed rectifier K^+ channel in nonmyelinated axons and amphibian nodes of Ranvier and has been used to determine their distribution[2-7]. In mammalian myelinated nerve K^+ channels are excluded from nodes[8-10]. Exposure of a mammalian internode in demyelination results in the appearance of outward K^+ currents and 4-AP sensitivity[11-17]. 4-AP prolongs action potentials in immature and regenerating mammalian nerve[12-14,16,18-20]. However, sensitivity to 4-AP does not localize K^+ channels to nodes; it indicates electrical and chemical accessibility. Immature and regenerating fibers may lack nodal K^+ channels and differ from mature nerve in the access of internodal channels to 4-AP. Potassium channels are also blocked by tetraethylammonium (TEA) and the relative effect of 4-AP and TEA varies. For example, TEA does not affect many 4-AP sensitive mammalian nonmyelinated or myelinated axons[20,21] and is relatively ineffective on internodal K^+ channels[12,13,22,23].

In animal models 4-AP restores conduction in demyelinated blocked fibers[12,15,24] and symptoms in multiple sclerosis (MS) are improved by 5–20 mg 4-AP[25,26]. Invertebrate axons and frog fibers have dissociation constants of 0.5 mM[5] and 10 μM[6,27], and 1-mM 4-AP is usually used to detect K^+ channels[16,17,19,20,26,28]. In MS patients plasma 4-AP levels were about 1 μM. The small amount of 4-AP needed could result from a higher sensitivity of internodal K^+ channels to 4-AP. Since K^+ currents and 4-AP access both vary, comparison of 4-AP sensitivity requires a system where nodal and internodal K^+ channels can be voltage-clamped, so a fiber serves as its own control. Nodal K^+ conductance in 16–18-μm frog fibers is 20–30% of the Na^+ conductance and is increased by lysolecithin (LPC). In 8–10-μm fibers there is no K^+ current unless LPC is used[1]. This diameter-dependence was used to compare K^+ channels. LPC-dependent channels were more sensitive to 4-AP than LPC-independent nodal channels, while TEA acts primarily on nodal channels. The size-dependent segregation of K^+ channels in frog fibers also allows a comparison of K^+ activation. While similar, there is a slight increase in the relative number of paranodal channels activated at low voltages compared to nodal channels.

Methods. Single 8–10-μm or 16–18-μm fibers of *Rana pipiens* were voltage-clamped in 115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 5 mM Tris[1,29,30]. Fiber ends were in isotonic KCl; pH was 7.30; and the temperature was 18–20 °C. Fibers were classed as sensory or motor based on the initial rate of decline of the action potential[31]. Fibers were held at −80 mV[32]. A 50-ms hyperpolarization was followed by 10-ms depolarizations to voltages between −40 mV and +100 mV. Leakage and capacity currents were determined for each fiber and subtracted during ionic current measurements. Currents were converted to conductances using experimentally determined values for the Na^+ equilibrium potential and a calculated value of −97 mV for the K^+ equilibrium potential[1,30,32]. Nodal and internodal resistances were measured[33] because nodal capacitance determines exposed membrane area and internodal resistance gives current. The Na^+ equilibrium potential was monitored because internal Na^+ blocks K^+ channels, leading to an apparent lack of K^+ conductance[34]. Data was used only if there was no change.