The Influence of Reserpine on Incorporation of ^{3}H-Thymidine into Mouse Liver DNA

Reserpine has been reported to cause marked inhibition of growth[1], especially of certain tumors[2,3]. Therefore, it was of interest to assert whether or not reserpine was able to interfere directly with the rate of DNA-synthesis. In order to answer this question we investigated, 60 h after the application of 2 mg/kg reserpine, the incorporation of ^{3}H-thymidine into liver DNA of female white mice adapted to an ambient temperature of 24°C or 32°C, respectively, because the body temperature of reserpinized mice was shown to be dependent to a high degree upon room temperature[4]. With regard to the reserpine-induced extreme anorexia, its influence on DNA synthesis was investigated in animals which were starved for 60 h.

Methods. All experiments were carried out on female white mice (20 g body wt.) of a laboratory bred NMRI-strain. The animals had free access to a standard diet (Altromin®) and tap water except for one group which was starved. One hour prior to killing, all animals (controls, reserpine-treated and 60-h-starved mice) were injected i.p. with a solution of 0.5 mCi/kg Thymidine-6-^{3}H (specific activity, 5 Ci/mmol, Radiochemical Center, Amersham). The livers were removed by dissection and weighed. The water content of the livers was determined by drying to constant weight at 110°C. DNA was isolated and DNA specific activity was measured as previously described[5]. Statistical evaluation of results was carried out using Student's *t*-test.

Results and discussion. As shown in the Table, 60 h after the administration of 2 mg/kg reserpine, body weight and liver weight were decreased by 26.4% and 31.8% at an ambient temperature of 24°C and by 20.2% and 23.7% at an ambient temperature of 32°C. After a starvation period of 60 h, body weight and liver weight were diminished by 42.8% or 46.6%, respectively. These results could be explained by the fact that the starved animals had a normal to increased motor activity and therefore a bigger energy consumption than animals reserpinized which were high-grade sedated. The water content of the livers (ml/g) neither changed in starvation nor after reserpine administration. In reserpinized animals, the rectal temperature remained unchanged at an ambient temperature of 32°C, but it was decreased by 5.5°C at 24°C room temperature. Starvation showed no significant influence on body temperature. The total amount of liver DNA showed no significant differences throughout all experiments (Table, row 4). As the amount of DNA/cell is constant, the decrease of the liver weight after starving and reserpine treatment might have been caused by a loss of cell components but not by a loss of cells. 60 h after the application of 2 mg/kg reserpine, the DNA specific radioactivity of the liver was decreased by 79.6% (24°C) or 78.9% (32°C), respectively, and by 46.9% 60 h after the beginning of starvation. In all experiments ^{3}H-thymidine was injected proportionally to body weight and not to the DNA content of the total liver, which was actually the same in reserpinized mice, starved animals and untreated controls (Table, row 4). Therefore, reserpinized mice received – in relation to DNA – 20.1% and 18.1%, respectively, and starved mice 44.4% less ^{3}H-thymidine than was injected to the controls (Table, row 5). So, it could be expected that a lower incorporation rate of ^{3}H-thymidine and a lower DNA specific radioactivity of the livers of reserpinized and starved animals would be found, in accordance with the smaller supply of ^{3}H-thymidine. The fact that ^{3}H-thymidine incorporation in starved animals was diminished by 46.9% corresponded to the smaller supply of ^{3}H-thymidine (–44.4%). Therefore it could be concluded that the incorporation of ^{3}H-thymidine into liver DNA actually was not altered in starvation. In reserpinized mice the DNA specific radioactivity was reduced by 79.6% or 78.9%, respectively, although they only received 20.1% and 18.1% less ^{3}H-thymidine than untreated controls. The incorporation rate of ^{3}H-thymidine into liver DNA of reserpinized mice was in fact reduced by nearly 60%. From these results it could be concluded that 2 mg/kg reserpine inhibited the incorporation of ^{3}H-thymidine into liver DNA. Reserpine must have a specific inhibitory effect on DNA synthesis which could not be explained by anorexia and hypothermia induced by reserpine. The inhibition of growth which was reported to be induced by reserpine[1,2] could therefore be explained by this inhibitory effect of reserpine on DNA synthesis.

	Body weight (g)	Rectal temperature (°C)	Liver weight (mg)	Total DNA of liver (mg/liver)	^{3}H-thymidine supply/DNA (μCi/mg DNA)	DNA specific radioactivity (cpm/mg DNA) $\times 10^{-3}$
Controls ($n = 12$)	20.8 ± 0.4	37.0 ± 0.1	1132 ± 39	2.68 ± 0.21	3.88	4.25 ± 0.24
Reserpine T = 24°C ($n = 12$)	15.3 ± 0.5	31.5 ± 0.7	771 ± 58	2.47 ± 0.21	3.10	0.87 ± 0.13
	(−26.4%)[a]	(−14.8%)[a]	(−31.8%)[a]	(−7.8%)	(−20.1%)	(−79.6%)[a]
Reserpine T = 32°C ($n = 12$)	16.6 ± 0.3	37.1 ± 0.2	863 ± 25	2.61 ± 0.09	3.18	0.90 ± 0.09
	(−20.2%)[a]	(+0.3%)	(−23.7%)[a]	(−2.6%)	(−18.1%)	(−78.9%)[a]
Starved ($n = 9$)	11.9 ± 0.4	37.9 ± 0.1	606 ± 34	2.75 ± 0.20	2.16	2.26 ± 0.31
	(−42.8%)[a]	(+2.4%)	(−46.6%)[a]	(+2.6%)	(−44.4%)	(−46.9%)[a]

All values represent mean values ± S.E.M. % deviation in parenthesis. [a] Significantly different from controls ($p = 0.05$ or less).

Zusammenfassung. Der Einbau von ^{3}H-Thymidin in die DNA der Leber von weiblichen weissen Mäusen wurde 60 h nach der Verabreichung von 2 mg/kg Reserpin und ausserdem 60 h nach Futterentzug an nicht reserpinisierten Tieren gemessen. Reserpin führt unabhängig von der unter dieser Substanz auftretenden Hypothermie und Anorexie zu einer Hemmung des ^{3}H-Thymidin-Einbaus in die DNS der Leber um etwa 60%.

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