Table II. Subcutaneous treatment of experimental rats with various substances

Substances (mg or ml/100 g body weight)	At zero h	After first treatment 1.5 h	3 h
1. Controls	–	–	–
2. Chlorpromazine[a]	1 mg/100 g	–	–
3. Chlorpromazine Succinate[b]	1 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g
4. Progesterone[c]	10 mg/100 g	–	–
5. Progesterone Succinate	10 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g
6. Chloramphenicol[d]	20 mg/100 g	–	–
7. Chloramphenicol Succinate	20 mg/100 g 0.2 ml/100 g	– 0.2 ml/100 g	– 0.2 ml/100 g

[a] Rats from group III and IV (Table I) are treated with CPZ in doses of 0.2 mg/100 g. [b] 0.4 *M* solution of disodium succinate. [c] Dissolved in 0.1 ml dioxane. [d] Introduced into stomach by stomach tube as aqueous suspension.

occurs and, hence, of ST oxidizing pathway, due to an intake of a comparatively great amount of glutathione (G-SH + GSSG). It seems that both types of glutathione may inhibit succindehydrogenase, GSSG directly, and G-SH after an oxidation[5]. The administration of ST into animals fed on a diet containing high levels of yeast and treated with agents inhibiting mainly the oxidation of NADH, led to a labilization of lysosomes. This effect was not observed when CPZ, progesterone and chloramphenicol were absent. Therefore, only when both oxidizing pathways were inhibited, ST might act as a lysosome labilizer. The results in Figure C indicate that the suggestion concerning the role of glutathione in the inversion of the effect of ST was confirmed. In addition, it was shown that this effect might be attained when other –SH and SS compounds are used (Figure D, E and F). At present, one can hardly say whether these compounds are the only cause for the effect observed, and whether the mechanism suggested is correct. There is no evidence that the presumed metabolic situations really exist in the living cells of experimental rats under the different conditions of nutrition and treatment described in this paper. If it is accepted on the basis of previous[1] and present results that, under the respective experimental conditions, there are disturbances in the functioning of ST and NADH oxiziding pathways, the degree of these disturbances, being undubtedly of great significance with a view to the manifestation of an effect, is fully unknown. The results in Figure C and D indicate that it is easier to reproduce labilization of lysosomes by ST when SH compounds are used. Depending on the dose of cystine, 2 types of action might be observed to be exerted by ST. When the rats received small amounts of cystine, ST in combination with CPZ increased lysosome labilizing action of CPZ, but when ST in combination with CPZ was applied to rats fed on a diet with great amount of cystine, an effect similar to that obtained on rats fed on ordinary diet was observed. To clear up this question, further experiments are necessary.

In this study only 2 batches of dried baker's yeast were used. It is not known, however, if all kinds of yeast would produce the same effect.

Zusammenfassung. Im Gegensatz zu Ratten bei Normaldiät, bei denen Sucinat einen Stabilisierungseffekt auf isolierte Leberlysosomen hat, vergrössert Succinat die Lysosomenpermeabilität bei Ratten mit einer Diät reich an Hefe oder ergänzt mit SH-Komponenten.

Ch. S. Popov

Higher Institute of Veterinary Medicine, Department of Chemistry and Biochemistry, Sofia 13 (Bulgaria), 29 April 1970.

[5] L. J. Webb, *Enzyme and Metabolic Inhibitors* (Publishing House 'Peace', Moskcow 1966), p. 585, in Russian.
[6] J. T. Dingle, I. M. Sharman and T. Moore, Biochem. J. *98*, 476 (1966).
[7] R. Gianetto and C. De Duve, Biochem. J. *59*, 433 (1955).

Renal Pressor Activity and Kidney Weight Responding to Angiotensin in Hypertensive Rats

Although angiotensin is known to cause a strong renal vasoconstriction[1,2], a loss of renal vasoconstrictor activity of angiotensin was observed during renal ischemia[3]. An increase[4,5] or a decrease[6] in juxtaglomerular cell granularity was observed in normal animals injected with angiotensin, but no similar observation was made in animals with experimental renal hypertension. The present study was undertaken to observe the changes in renin content and in granularity of the juxtaglomerular apparatus of an ischemic kidney exposed in vivo to long-acting angiotensin.

Material and methods. 58 female rats of Wistar-King strain weighing approximately 100 g were used. Left renal artery clipping was achieved with and without accompaniment of right nephrectomy, in 17 and 18 rats, respectively. For the respective control, right nephrectomy was done in 14 rats and no nephrectomy in 9 rats. All rats were fed a commercial rat diet (production of Oriental Yeast Manufacturing Co., Japan) and tap water ad libitum. Blood pressure was determined at weekly intervals by tail sphygmography following the surgery. In the sixth week the rats were injected s.c. either with 10 µg of synthetic angiotensin II-asp[1]-β-amide (Hypertensin, CIBA) suspended in 0.2 ml of sesame oil or with 0.2 ml of sesame oil once everyday for 6 days and sacrificed. The 58 rats were thus divided into 7 groups as follows: group I, right nephrectomy + oil (6 rats); group II, right nephrectomy + angiotensin in oil (8 rats); group III, right nephrectomy + left renal artery clipping + oil (9 rats); group IV, right nephrectomy + left renal artery clipping + angiotensin in oil (8 rats); group V, both kidneys untouched + angiotensin in oil (9 rats); group VI, left renal artery clipping + oil (9 rats); group VII, left renal artery clipping + angiotensin in oil (9 rats). When sacrificed, heart and kidney were weighed. 2 coronal sections of the kidney were made through the hilus at a distance of about 1.5 mm and the middle section was processed for the determination of juxtaglomerular granular index (JGI) according to the method of Hartroft and Hartroft[7]. Renal renin content was deter-

Effect of angiotensin II administration on renal pressor activity and kidney weight in the rats made hypertensive by main renal artery constriction

Group No.	Procedure	No. of rats	Blood pressure[a] (mmHg)	Renin content (angiotensin formation) μg/g of kidney Lt	Rt	μg/each kidney Lt	Rt	JGI Lt	Rt	Kidney/body weight (%) Lt	Rt	Heart/body weight (%)
I	Uninephrectomy + oil	6	135.7 ± 2.6	1.22 ± 0.19		1.61 ± 0.29		18.8 ± 2.8		0.88 ± 0.051		0.37 ± 0.028
II	Uninephrectomy + angiotensin in oil	8	149.5 ± 5.3	1.47 ± 0.19		1.78 ± 0.20		17.0 ± 1.2		0.84 ± 0.047		0.37 ± 0.013
III	Uninephrectomy renal artery clipping + oil	9	189.6 ± 8.4	1.29[b] ± 0.29		1.42[c] ± 0.38		16.6[b] ± 2.1		0.75[b] ± 0.052		0.51 ± 0.044
IV	Uninephrectomy renal artery clipping + angiotensin in oil	8	196.0 ± 17.7	0.48[b] ± 0.13		0.60[c] ± 0.16		7.3[b] ± 1.2		0.93[b] ± 0.057		0.51 ± 0.020
V	+ Angiotensin in oil	9	114.2 ± 3.9	2.36 ± 1.06	2.48 ± 1.53	0.30 ± 0.16	0.29 ± 0.23	12.4 ± 1.1	13.3 ± 1.6	0.39 ± 0.009	0.38 ± 0.007	0.40 ± 0.006
VI	Renal artery clipping + oil	9	188.2 ± 7.7	10.73 ± 5.63	0.70 ± 0.42	1.08 ± 0.61	0.096 ± 0.056	42.4 ± 4.5	3.9 ± 0.8	0.41 ± 0.012	0.56 ± 0.039	0.53 ± 0.019
VII	Renal artery clipping + angiotensin in oil	9	188.7 ± 7.5	4.13 ± 2.03	0.81 ± 0.086	0.40 ± 0.22	0.15 ± 0.011	31.1 ± 4.1	2.3 ± 0.5	0.37 ± 0.018	0.48 ± 0.014	0.50 ± 0.018

The values represent mean ± S.E. Lt, left kidney; Rt, right kidney. [a] Blood pressure determined before sacrifice by tail sphygmography. [b] $P < 0.05$. [c] $0.1 > P > 0.05$.

mined as follows. The kidney tissue of upper half section was ground with cold physiological saline in the ratio of 30 volumes per 1 volume of kidney tissue. Equal volume of the saline homogenate and renin substrate (plasma from 24 h nephrectomized rat) were incubated with EDTA (5×10^{-3}mol) at pH 6.5, 37 °C for 10 min. The incubation mixture was then boiled and centrifuged. Pressor activity of the supernatant was assayed by a pentolinium-treated rat anesthetized with amytal soda (8–10 mg/100 g body weight, s.c.); the activity was expressed as angiotensin II formation per gram of kidney tissue and per each kidney.

Results. The results were summarized in the Table. Average of blood pressure in the rats with clipped kidney rose within one week following the surgery and reached 189.6 mmHg, 196.0 mmHg, 188.2 mmHg and 188.7 mmHg in groups III, IV, VI and VII, respectively, when sacrificed. It remained within normal limits throughout the experiment in groups I, II and V, and was 135.7 mmHg, 149.5 mmHg and 114.2 mmHg, respectively, but it was somewhat higher in groups I and II (uninephrectomized) than in group V (without nephrectomy). Although blood pressure was somewhat higher in the groups injected with angiotensin than in those with oil, the difference between groups I and II, III and IV, or VI and VII was not statistically significant. Heart weight was not affected by the administration of angiotensin.

Renal renin content was 1.22, 1.47, 1.29 and 0.48 μg/g of kidney tissue (as angiotensin II formation) and 1.61, 1.78, 1.42 and 0.60 μg per each kidney, and JGI 18.8, 17.0, 16.6 and 7.3 for the uninephrectomized groups of I, II, III and IV, respectively. Both of renal renin content per g of kidney tissue and JGI were definitely reduced in group IV compared with those in group III, and the difference was statistically significant ($P < 0.05$). The difference of renin content per each kidney between the 2 groups was not statistically significant ($0.1 > P > 0.05$). They were not substantially different between groups I and III, showing that pressor activity of clipped kidney remained at the same level as that of the non-clipped control kidney, when the rats were not given angiotensin. Kidney weight was 0.88, 0.84, 0.75 and 0.93% of body weight for groups I, II, III and IV, respectively, which indicated that weight of the non-clipped kidney was not affected by angiotensin but of the clipped kidney significantly increased ($P < 0.05$).

Renin content and JGI were increased of left kidney with renal artery clipping and decreased of right untouched kidney as indicated by the values for groups VI and VII compared with those for group V. Both of them were somewhat reduced in group VII (angiotensin) compared with those in group VI (no angiotensin), but the difference of each between the 2 groups was not statistically significant because of a wide variation of the values determined. Weights of both kidneys in group VII were not significantly affected by angiotensin.

Discussion. The study clearly demonstrate that both renal pressor activity and kidney weight were not significantly affected by angiotensin in non-clipped

[1] N. S. ASSALI and A. WESTERNSTEN, Circulation Res. *9*, 189 (1961).
[2] M. J. MANDEL and L. A. SAPIRSTEIN, Circulation Res. *10*, 807 (1962).
[3] J. C. MCGIFF and H. D. ITSKOVITZ, J. clin. Invest. *43*, 2357 (1964).
[4] Y. J. KATS, P. R. PATEK and S. BERNICK, Circulation Res. *11*, 955 (1962).
[5] S. KAMURA, T. NIWA, F. R. SKELTON and L. L. BERNARDIS, Experientia *22*, 547 (1966).
[6] H. YOSHIDA, S. YOKOII, O. AOZI, M. NAITO, T. KIMURA and H. KIMURA, Jap. J. Nephrol. *9*, 63 (1967).
[7] P. M. HARTROFT and W. S. HARTROFT, J. exp. Med. *97*, 415 (1953).

control kidneys, but the former markedly reduced and the latter increased of the clipped kidneys in uninephrectomized rats. The same effect was not clearly observed in the rats bearing untouched contralateral kidney. A decrease in renal pressor activity of the clipped kidney elicited by angiotensin in the rat was rather controversial to our previous observation made in the dog that renin release from the clipped kidney was augmented by infusion of very small amount of angiotensin[8]. The discrepancy may have been caused not only by a large difference of dose of angiotensin administered but also of duration of the action of this substance between the two experiments[9,10].

Zusammenfassung. Angiotensin erniedrigte nur bei ischaemischen Nieren die Reninproduktion und erhöhte parallel dazu das Nierengewicht. Die Angiotensinwirkung ist somit abhängig von der Stoffwechsellage der Niere.

K. Tanaka, Y. Morotomi,
K. Kumamoto and T. Omae

Second Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka (Japan), 3 July 1970.

[8] K. Tanaka, T. Omae, N. Hattori and S. Katsuki, Jap. Circulation J. *33*, 235 (1969).

[9] We are indebted to CIBA, Basel (Switzerland) for their generous supply of synthetic angiotensin II.

[10] Supported in part by research grant from Japanese Ministry of Education.

Visual Unpatterned Input Determines the Occurrence of Reward-Contingent Positive Variation

Cats deprived of food and trained to press a lever for 1 cm^3 of milk reward, display bursts of high amplitude (180–200 μV) α-activity of 5 to 9 c/sec over the primary and secondary cortical visual projections. This phenomenon triggered by consumption of reward, and termed postreinforcement synchronization (PRS)[1], recorded with reference to the skull, frontal cortex or subjacent white matter, is always associated with a transient (3–5 sec duration) and abrupt surface positive steady potential shift of 200 to 400 μV over the same cortical region[2,3]. The positive shift[2,3], like the PRS[1,4], depends not only on the presence of light but also on the relative taste and appropriateness of food reward; therefore, the steady potential shift was termed 'reward contingent positive variation' (RCPV)[2]. The question was raised whether the RCPV and PRS responses depend upon visual perception of reward (and possibly of the environment which might have acquired conditional properties) or upon a diffuse visual input devoid of any conditional and informational properties. The following experiments were designed to answer this question.

Material and methods. A total of 48 experimental trials were carried out in 6 adult cats of either sex, trained to press a lever for 0.8 ml of milk reward presented on a schedule such that pressing a lever produced the reward aperiodically, approximately once every 4 or 10 sec. All subjects were kept on 23 h water and food deprivation schedule while being trained to press the lever until satiation, 5 days a week for 3 to 6 months. Approximately 80% of the training sessions were conducted in the absence of light. During the 4 weeks prior to this experimental paradigm, the subjects were allowed to perform only in the dark in order to induce extinction of possible conditional responses to light and perceived environment. Under pentobarbital anesthesia, 3–10 solid-type, nonpolarizable Ag-AgCl electrodes[5] were implanted over the parieto-occipital cortex. Reference electrodes were implanted in the subjacent white matter 3 mm below the surface for transcortical recording which was found free from any discernible influences caused by directional changes in the standing corneo-retinal potential associated with eye movements[3]. Lapping was monitored by using the milk delivery cup converted to a drinkometer[6]. The technique of recording and integration of RCPV responses was previously described[2]. The integrating system was calibrated to produce 2 pen deflections 30 mm each in response to a 100 μV positive shift lasting 1 sec. This value was arbitrarily accepted as one unit of RCPV triggered by 0.8 ml of milk reward. During the control trials each subject was allowed to obtain 40–60 milk rewards in an illuminated test chamber (the intensity of light was set at approximately 26 cd/m^2) and, subsequently, 30–50 rewards in the dark. Afterwards, this sequence was repeated. The control trials were alternated with the experimental ones, during which the subject was allowed to perform while its patterned vision was prevented by either a pair of translucent contact lenses or goggles. Statistical differences between the integrated average RCPV responses were determined by Student's *t*-test.

Results and discussion. All 6 subjects showed well-developed PRS and RCPV responses, but only in the presence of light, even though their patterned vision was prevented. After 2–4 trials, 75–80% of the averaged integrated RCPV responses (each based on 10 single integrated RCPVs) obtained while patterned vision was prevented were not statistically different from those obtained during control trials, i.e., while patterned vision was allowed ($P > 0.05$). A total 2900 consumptions of milk rewards in the dark were considered, and none of the subjects showed a single PRS or RCPV response, provided that all sources of light have been carefully eliminated. During a prolonged performance in the dark, usually a variant of PRS developed which was described

[1] M. B. Sterman, S. R. Roth and C. D. Clemente, Fedn Proc. *22*, 399 (1963). – C. D. Clemente, M. B. Sterman and W. Wyrwicka, Electroenceph. clin. Neurophysiol. *16*, 355 (1964). – N. A. Buchwald, F. E. Horwath, E. J. Wyers and C. Wakefield, Nature, Lond. *201*, 830 (1964). – S. R. Roth, M. B. Sterman and C. D. Clemente, Electroenceph. clin. Neurophysiol. *23*, 509 (1967). – T. J. Marczynski, A. J. Rosen and J. T. Hackett, Electroenceph. clin. Neurophysiol. *24*, 227 (1968).

[2] T. J. Marczynski, J. L. York and J. T. Hackett, Science *163*, 301 (1969).

[3] T. J. Marczynski, J. T. Hackett, C. J. Sherry, J. H. Rick and J. L. York, Brain Res., in press. – T. J. Marczynski, J. T. Hackett, C. J. Sherry and Sharon Allen, Brain Res., in press.

[4] M. B. Sterman and W. Wyrwicka, Brain Res. *6*, 143 (1967).

[5] H. W. Bond and P. Ho, Electroenceph. clin. Neurophysiol. *28*, 206 (1970).

[6] The conversion of the milk delivery cup into a drinkometer was accomplished by feeding a continuous 50 c/sec train of impulses from a S-8 Grass stimulator into the milk container, and by subsequently recording this signal from one of the skull electrodes with reference to the 'common ground' every time the subject's tongue touched the cup.