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WATER BALANCE EFFECTS OF SYSTEMIC AND INTRACEREBROVENTRICULAR ADMINISTRATION OF ANGIOTENSIN II IN THE TOAD *BUFO ARENARUM*

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Abstract—1. Systemic administration of angiotensin II (10 μg/kg) to fully hydrated toads decreased urine production but did not affect natripheric or hydrosmotic water fluxes across the skin.

2. The antidiuretic response was not modified by the simultaneous α-adrenergic blocking with tolazoline (5 mg/kg).

3. In a similar way, single intracerebroventricular injections of 1 μl 10^{-4}M angiotensin II, decreased urine production but did not affect water uptake.

4. Finally, systemic injections of the angiotensin blocker salarasin (100 μg/kg) did not affect the cutaneous hydrosmotic response of dehydrated toads.

5. We conclude that angiotensin II has no effects on water uptake in this species. The antidiuretic response to angiotensin II, and the involvement of adrenergic mechanisms are discussed.

INTRODUCTION

Intravenous or intraperitoneal injections of angiotensin II triggers drinking behaviour in fishes (Hirano *et al.*, 1978; Takei *et al.*, 1979; Malvin *et al.*, 1980; Carrick and Balment, 1983; Kobayashi *et al.* 1983; Beasley *et al.*, 1986), reptiles (Fitzsimons and Kaufman, 1977), birds (Evered and Fitzsimons, 1976, 1981; Snapir *et al.*, 1977; Takei, 1977; Schwob and Johnson, 1977) and mammals (Fitzsimons and Simons, 1969; Epstein and Hsiao, 1975; Trippodo *et al.*, 1976). The intracerebral injection of angiotensin II in doses much lower than the systemic ones, induces drinking in birds (Wada *et al.*, 1975; Evered and Fitzsimons, 1976; Snapir *et al.*, 1976; Schwob and Johnson, 1977) and mammals (Epstein *et al.*, 1970 for review see Fitzsimons, 1979) suggesting the existence of an angiotensin dipsogenic receptor in the forebrain of vertebrates.

Dehydrated amphibians normally do not drink, but rehydrate by absorbing water across their permeable skin, a process that can be considered as "cutaneous drinking" (Bentley and Yorio, 1979). Some amphibians appear to seek water in which to lie in order to rehydrate. It has been suggested that this process may constitute a primeval thirst (Fitzsimons, 1975) which is slaked by the absorption of water through the integument, instead of more conventional oral drinking.

There is good evidence of the existence of a renin-angiotensin system in several species of amphibians (Wilson, 1984 for a review). However, the role of angiotensin II in water intake and water balance is not clear. Intravenous angiotensin II does not produce drinking in frogs (Hirano *et al.*, 1978) and intraperitoneally injected aniotensin II fails to stimulate the ingestion of water in tadpoles and newts (Kobayashi *et al.*, 1979). However, it has been found that isolated toad skins present an angiotensin-dependent increase in osmotic water prermeability, short-circuit current and cyclic AMP levels (Coviello and Brauckmann, 1973; Coviello *et al.*, 1976; 1978; Soria *et al.*, 1987).

In *Bufo arenarum*, a renin-like enzyme in kidney, an angiotensin-like substance in plasma (Nolly and Fasciolo, 1971) and an angiotensin converting enzyme in serum, kidney and skin (Fernandez-Pardal *et al.*, 1986) have been found. The aim of this study is to evaluate the involvement of the renin angiotensin system in the water balance control in this species.

MATERIALS AND METHODS

Animals

Adult male toads (*Bufo arenarum*) weighing 80–150 g were collected near Buenos Aires and used after 2–3 weeks of captivity. During this period they were kept in large cages with free access to tap-water and fed once a week with ox liver.

Water balance measurements in hydrated toads

Animals were kept in opaque individual plastic boxes (12 × 12 × 8 cm) containing 150 ml of distilled water for 24 hr to ensure hydration. At this time their urinary bladders were emptied by inserting a glass probe into the cloaca and applying suprapubic pressure, and the standard weight (the weight of the hydrated animal with its bladder empty, Ruibal, 1962) was recorded. The animals were then kept in distilled water for another 2 hr. During this time they were weighted every 15 min before and after bladder emptying. The gross weight gain over each period was assumed to be due to water uptake, whereas the weight loss produced by emptying the bladder was assumed to be due to the urine production during that period.

In the experiments on peripheral administration, at the end of the first hour, the animals were injected with 115 mM NaCl, Asn^1, Val^5 angiotensin II (Sigma) 10 μg/kg, tolazoline (Sigma) 5 mg/kg or angiotensin II (10 μg/kg) plus tolazoline

(5 mg/kg). The drugs were dissolved in 115 mM NaCl and the solutions were administrated through the dorsal linfatic sac. In all groups the volume injected was 1 ml/kg of body weight.

In the experiments of central administration, at the end of the first hour, the animals received 1 μl of 115 mM NaCl or 10^{-4} M angiotensin II. The hormone was dissolved in 115 mM NaCl and the solutions were injected with a 5 μl Hamilton syringe through a stainless steel cannula which had previously been implanted stereotaxically into the third ventricle of the brain.

The angiotensin II used both in systemic and in intracerebroventricular experiments was homologous to the amphibian (*Rana catesbeiana*) endogenous peptide (Hasewawa *et al.*, 1983).

Water uptake measurements in dehydrated toads

The toads ($n = 10$) were dehydrated to 79.89 ± 0.54 per cent of their standard weight. During dehydration the animals were kept in individual open containers (mesh 2 cm) at room temperature. In these conditions they lost 20% of their body weight in the course of 24–48 hr.

Fifteen minutes before rehydration started, the animals were injected with 115 mM NaCl or Salarasin (Sar^1, Val^5 Angiotensin II, Sigma) 100 μg/kg through the dorsal linfatic sac.

The toads were rehydrated in opaque individual plastic boxes containing 150 ml of distilled water for 3 hr. During this period they were weighed every 15 min in a Ohaus Dial-O-Gram balance with a precision of 0.1 g. The weight gain over each period was assumed to be due to water uptake and expressed as ml/100 g/hr. Generally, toads do not urinate during rehydration. However, the animals which showed body weight decrease at any time during the experiments were excluded from the analysis.

In all cases, rehydration experiments were made between 48 and 72 hr after dehydration started.

Surgery

The animals were anesthetized by exposure to ether. The skin on the frontoparietal bone was removed and a hole (o.d. = 0.5 mm) was made on the sagital plane 2 mm ahead of the line that passes through the auditory capsules. Then, by means of a micromanipulator a stainless steel cannula (0.28 mm external bore, 1 cm long, 0.1 μl inner volume) was lowered 3.5 mm deep from the upper surface of the bone. The cannula was cemented into place with dental acrylic attached to the skull with a jeweller's screw (o.d. = 0.8 mm). The animals were allowed to recover from the surgery at least 72 hr before testing began.

Analysis

All the results are presented as mean $\pm$ SE of the mean. In experiments with hydrated animals, within group comparisons were made using a paired t-test (2 tail) whereas in experiments with dehydrated animals, between groups comparisons were made using 2-way Analysis of Variance for repeated measures.

RESULTS

The systemic administration of 10 μg/kg of angiotensin II to fully hydrated toads kept in distilled water decreased the urine production ($P < 0.02$) but did not modify ($P < 0.34$) the water uptake across the skin (Fig. 1). When the toads were kept in 23 mM NaCl, angiotensin II again did not produce any changes in water uptake ($P < 0.65$) and the decrease in urine production ($P < 0.05$) was similar to the observed in the group kept in distilled water.

To evaluate the role of catecholamines in this antidiuretic response, the α-adrenergic blocker tol-

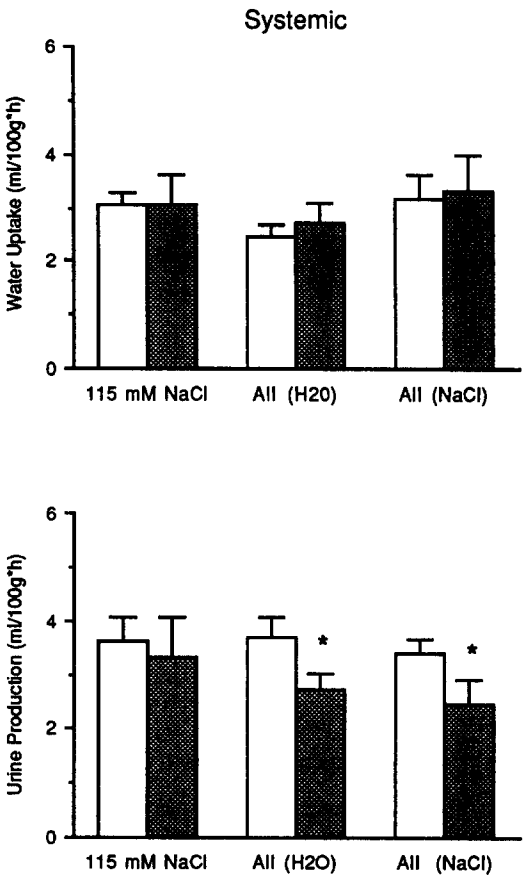

Fig. 1. Water uptake across the skin and urine production before (empty bars) and after (filled bars) the systemic administration of 115 mM NaCl ($n = 4$) or angiotensin II (10 μg/kg). To evaluate the effects of angiotensin II on hydrosmotic and natripheric water fluxes the hormone was assayed in toads kept in distilled water ($n = 11$) or in 23 mM NaCl ($n = 5$). *$2P < 0.05$.

azoline was injected simultaneously with angiotensin II. Tolazoline plus angiotensin II decreased the urine production ($P < 0.05$) in a similar way to angiotensin II (Fig. 2). Besides, the increase in water uptake produced by tolazoline ($P < 0.02$) was reduced by the simultaneous angiotensin II administration ($P < 0.36$).

On the other hand, angiotensin II (1 μl, 100 μg/ml) injected intracerebroventricularly produced a decrease ($P < 0.003$) in urine production but again was not effective in increasing water uptake ($P < 0.48$) (Fig. 3). There were no observed changes in water uptake either at the biggest dose assayed (1 μl, 1 mg/kg). In this case, water uptake before and after intracranial injection was 3.79 ± 0.49 and 3.30 ± 0.33 ml/100 g/hr ($n = 5$, $P < 0.38$). No volumetric effects on urine production ($P < 0.91$) and water uptake ($P < 0.48$) were observed in the control group injected intracerebroventricularly with 1 μl of 115 mM NaCl (Fig. 3).

To evaluate the involvement of angiotensin II in the cutaneous hydrosmotic response, toads dehydrated to $79.89 \pm 0.54\%$ of their standard weight were injected with the competitive angiotensin

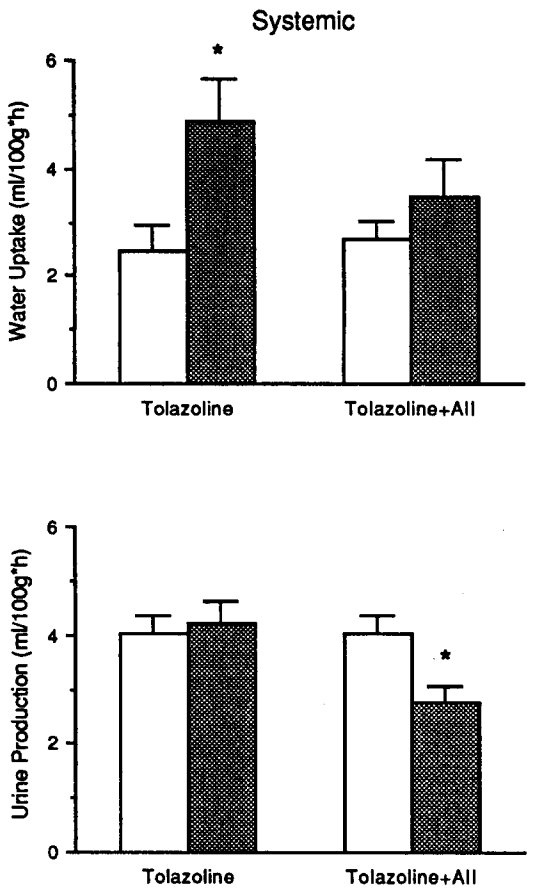

Fig. 2. Water uptake across the skin and urine production before (empty bars) and after (filled bars) the systemic administration of tolazoline 5 mg/kg ($n = 6$) or tolazoline 5 mg/kg plus angiotensin II 10 μg/kg ($n = 5$). *$2P < 0.05$.

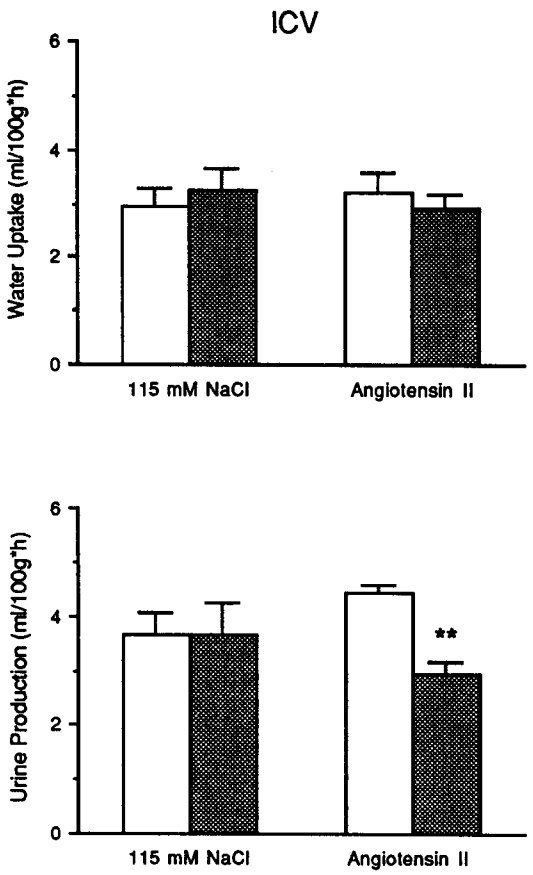

Fig. 3. Water uptake across the skin and urine production before (white bars) and after (dotted bars) the intracerebroventricular injection of 1 μl of 115 mM NaCl ($n = 4$) or 10^{-4} M angiotensin II ($n = 6$). **$2P < 0.01$.

blocker salarasin 15 min before the rehydration began (Fig. 4). No significant differences in water uptake values ($P < 0.59$) between the control (115 mM NaCl) and the experimental (salarasin 100 μg/kg) groups were observed during the 3 hr of rehydration.

DISCUSSION

Our results suggest that systemic treatment with exogenous angiotensin II produces a catecholamine-independent antidiuretic response. Angiotensin II-induced catecholamine release has been shown in amphibians and other vertebrates (Carroll and Opdyke, 1982). Adrenaline and α-adrenergic agonists affect renal function, producing antidiuresis (Gallardo *et al.*, 1980; Reboreda and Segura, 1988). However, the angiotensin II-induced antidiuretic response was not affected by the simultaneous administration of the α-adrenergic blocker tolazoline, thus suggesting that it is not mediated by catecholamines.

With reference to the glomerural or tubular effects of systemically injected angiotensin II, in previous experiments in *Bufo arenarum*, it was observed that the intravenous injection of this hormone in doses between 0.5–5 μg/kg produced a long-lasting (10–20 min) hypertension. Although this result would indicate that the angiotensin II antidiuretic response has a glomerular component, possible tubular effects of this hormone cannot be rejected.

In addition, intracerebroventricular injection of angiotensin II in doses 10 (1 μl, 100 μg/ml) and 100

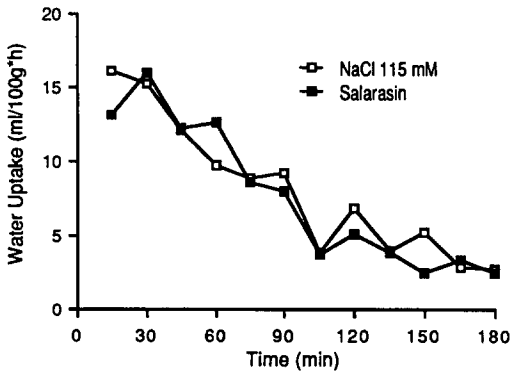

Fig. 4. Changes in water uptake across the skin during 3 hr of rehydration in toads dehydrated to 80% of their standard weight. The toads were injected 15 min before the rehydration started with 115 mM NaCl ($n = 5$) or salarasin 100 μg/kg ($n = 5$).

(1 μl, 10 μg/ml, data not shown) times lower than systemic doses produced a similar antidiuretic response. This result suggests that the mechanism involved must be different, since the doses used in the intracranial experiments have no effect if injected systemically. In that respect, an angiotensin II-induced vasotocin release similar to the one shown in mammals (Keil *et al.*, 1975) could be involved.

Angiotensin II injected both systemically and intracerebroventricularly did not increase water uptake in toads kept in distilled water. Furthermore, changes in water uptake when toads were kept in 23 mM NaCl were not observed either. These results indicate that angiotensin II has no effect on hydrosmotic and natripheric water fluxes across the skin.

Further evidence suggesting that angiotensin II is not involved in the control of water skin permeability is the failure of salarasin to modify the cutaneous hydrosmotic response in dehydrated animals. Water uptake across the skin increases between 4 and 6 times in 20% dehydrated toads as a consequence of the increase in plasmatic osmolarity and skin permeability. This cutaneous hydrosmotic response is not modified by the angiotensin II antagonist, salarasin, as it could be expected if the skin permeability were modulated by angiotensin.

As it was mentioned before, dehydrated amphibians seek water in which they rehydrate and this behaviour could be controlled by angiotensin II. However, toads (*Bufo arenarum*) kept in a terrarium with free access to water and injected intracerebroventricularly with angiotensin II (1 μl, 100 μg/ml) did not show any differences in latency or time spent in water from the control group (unpublished data).

We conclude that angiotensin II has no effects neither on water uptake nor on drinking behaviour in this amphibian species. In that respect, mechanisms controlling "cutaneous drinking" in amphibians appear to be different from the ones in other vertebrates.

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REFERENCES

Beasley D., Shier D. N., Malvin R. L. and Smith G. (1986) Angiotensin-stimulated drinking in marine fish. *Am. J. Physiol.* **250,** R1034–R1038.

Bentley P. J. and Yorio T. (1979) Do frogs drink? *J. exp. Biol.* **79,** 41–46.

Carrick S. and Balment R. J. (1983) The renin-angiotensin system and drinking in the euryhaline flounder. *Platichthys flesus. Gen. comp. Endocrinol.* **51,** 423–433.

Carroll R. G. and Opdyke D. F. (1982) Evolution of angiotensin II-induced catecholamine release. *Am. J. Physiol.* **243,** R65–R69.

Coviello A. and Brauckmann E. S. (1973) Hydrosmotic effect of angiotensin II in isolated toad skin. *Acta physiol. latinoam.* **23,** 18–23.

Coviello A., Elso G. and Fernandez F. M. (1976) Effect of angiotensin II on short circuit current in amphibian membranes. *Biochem. Pharmacol.* **25,** 106–107.

Coviello A., Raisman R., Elso G. and Orce G. (1978) Role of cyclic AMP and angiotensin II in the response of toad skin to angiotensin II. *Biochem. Pharmacol.* **27,** 611–612.

Epstein A. N. and Hsiao S. (1975) Angiotensin as a dipsogen. In *Control Mechanisms of Drinking* (Edited by Peters G., Fitzsimons J. T. and Peters-Haefeli L. L.), pp. 108–116. Springer, Berlin.

Epstein A. N., Fitzsimons J. T. and Rolls B. J. (1970) Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210,** 457–474.

Evered M. D. and Fitzsimons J. T. (1976) Drinking induced by angiotensin in the pigeon *Columba livia. J. Physiol.* **263,** 193P–194P.

Evered M. D. and Fitzsimons J. T. (1981) Drinking and changes in blood pressure in response to angiotensin II in the pigeon, *Columbia livia. J. Physiol.* **310,** 337–352.

Fernandez-Pardal J., Perseguino J. C., Berman D. M. and Coviello A. (1986) Angiotensin converting enzyme in the toad *Bufo arenarum. Comp. Biochem. Physiol.* **83A,** 331–332.

Fitzsimons J. T. (1975) Thirst and sodium appetite in the regulation of body fluids. In *Control Mechanisms of Drinking* (Edited by Peters G., Fitzsimons J. T. and Peters-Haefeli L. L.), pp. 1–7. Springer, Berlin.

Fitzsimons J. T. (1979) *The Physiology of Thirst and Sodium Appetite.* Monographs of the Physiological Society 35. Cambridge University Press, Cambridge.

Fitzsimons J. T. and Simons B. J. (1969) The effect on drinking in the rat of intravenous angiotensin, given alone or in combination with other stimuli of thirst. *J. Physiol.* **203,** 45–57.

Fitzsimons J. T. and Kaufman S. (1977) Cellular and extracellular dehydration and angiotensin as stimuli to drinking in the common iguana *Iguana iguana. J. Physiol.* **265,** 443–463.

Gallardo R., Pang P. K. T. and Sawyer W. H. (1980) Neural influences on bullfrog renal function. *Proc. Soc. Exp. Biol. Med.* **165,** 223–240.

Hasegawa Y., Watanabe T. X., Sokabe H. and Nakajima T. (1983) Chemical structure of angiotensin in the bullfrog *Rana catesbeiana. Gen comp. Endocrinol.* **50,** 75–80.

Hirano T., Takei Y. and Kobayashi H. (1978) Angiotensin and drinking in the eel and frog. In *Osmotic and Volume Regulation* (Edited by Jorgensen C. B. and Skadhauge E.), pp. 123–134. Alfred Benzom Symposium XI. Academic Press, New York.

Keil L. C., Summy-Long J. and Severs W. B. (1975) Release of vasopressin by angiotensin II. *Endocrinology* **96,** 1063–1065.

Kobayashi H., Uemura H., Takei Y., Itatsu N., Osawa M. and Ichinohe K. (1983) Drinking induced by angiotensin II in fishes. *Gen comp. Endocrinol.* **49,** 295–306.

Kobayshi H., Uemura H., Wada M. and Takei Y. (1979) Ecological adaptation of angiotensin II-induced thirst mechanisms in tetrapods. *Gen. comp. Endocrinol.* **38,** 93–104.

Malvin R. L., Shiff D. and Eiger S. (1980) Angiotensin and drinking rates in the heuryhaline killfish. *Am. J. Physiol.* **239,** R31–R34.

Nolly H. and Fasciolo J. C. (1971) The renin-angiotensin system in *Bufo arenarum* and *Bufo paracnemis. Comp. Biochem. Physiol.* **39A,** 823–831.

Reboreda J. C. and Segura E. T. (1988) Control endocrino y nervioso del balance acuoso en el anfibio anuro *Bufo arenarum. Acta Zool. Lilloana* (in press).

Ruibal R. (1962) The adaptative role of bladder water in the toad *Bufo cognatus. Physiol. Zool.* **35,** 218–223.

Schwob J. E. and Johnson A. K. (1977) Angiotensin-induced dipsogenesis in domestic fowl (*Gallus gallus*). *J. comp. Physiol. Psychol.* **91,** 182–188.

Snapir J. E., Robinson B. and Goldschalk M. (1976) The drinking response of the chicken to peripheral and central administration of angiotensin II. *Pharm. Biochem. Physiol.* **5,** 5–10.

Soria M. O., Berman D. M. and Coviello A. (1987) Com-

parative effects of angiotensin II on osmotic water permeability in the toad (*Bufo arenarum*). *Combp. Biochem. Physiol.* **86A,** 147–150.

Takei Y. (1977) Angiotensin and water intake in the Japanese quail (*Coturnix coturnix japonica*). *Gen comp. Endocrinol.* **31,** 364–372.

Takei Y., Hirano T. and Kobayashi H. (1979) Angiotensin and water intake in the japanese eel, *Anguilla japonica*. *Gen comp. Endocrinol.* **38,** 466–475.

Trippodo N. C., McCaa R. E. and Guyton A. C. (1976) Effect of prolonged angiotensin II infusion on thirst. *Am. J. Physiol.* **230,** 1063–1066.

Wada M., Kobayashi H. and Farner D. S. (1975) Induction of drinking in the white-crowned sparrow, *Zonotrichia leucophris gambelii*, by intracranial injection of angiotensin II. *Gen comp. Endocr.* **26,** 192–197.

Wilson J. X. (1984) The renin-angiotensin system in non-mammalian vertebrates. *Endocr. Rev.* **5,** 45–61.