Pharmacology Biochemistry & Behavior, Vol 29, pp 547–551 © Pergamon Press plc, 1988 Printed in the U S A 0091-3057/88 $3 00 + 00

Feeding and Drinking Behaviour Following Angiotensin Converting Enzyme Blockade: Role of Injectant pH

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Received 2 December 1986

DI NICOLANTONIO, R AND R S WEISINGER *Feeding and drinking behaviour following angiotensin converting enzyme blockade Role of injectant pH* PHARMACOL BIOCHEM BEHAV **20**(3) 547–551, 1988 —Angiotensin converting enzyme (ACE) is a circulating dipeptidase which has a broad specificity and is known to metabolise a range of circulating peptides While a number of circulating peptides are believed to modulate food intake, it is not known if ACE plays a role in the control of feeding behaviour and therefore in this study we have examined the effect of the potent, specific ACE antagonists captopril (SQ 14225) and enalapril (MK421) on food and water intake following food deprivation and 2-deoxyglucose treatment in the rat One hour captopril (50 mg/kg, IP) pretreatment significantly reduced the food intake of 24 hr food deprived rats Because captopril solutions have a low pH (2 0), the effect of buffered captopril on food intake following 24 hr food deprivation was also examined Buffered captopril also significantly reduced the food intake of 24 hr food deprived rats, but not to the same extent as unbuffered captopril Naloxone pretreatment (1 mg/kg, IP) did not antagonize the effect of captopril on food intake indicating that the anorexic action of captopril was not due to alterations in opiate peptide levels Buffered captopril did not reduce the food intake of food-replete rats receiving 2-deoxyglucose (300 mg/kg, IP) or alter blood gases or pH However, an equimolar buffered dose of the structurally different ACE inhibitor enalapril failed to significantly alter food deprivation-induced food intake, suggesting that this action of captopril in reducing food intake was unrelated to ACE blockade In view of these findings a re-examination was made of the effect of captopril on dehydration-induced drinking where it was found that while unbuffered captopril reduced the water intake of 24 hr dehydrated rats the buffering of the captopril abolished this effect These findings do not suggest a role for ACE in the control of feeding behaviour but do indicate that it is important to consider the effect of the pH of captopril solution when studying its effects on behaviour

Angiotensin Captopril Enalapril Hunger Ingestive behaviour Thirst 2-Deoxyglucose

FEEDING behaviour is a complex process involving central nervous system structures, circulating hormones and gastrointestinal function (see reviews [16,20]) Circulating peptides such as cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and opiates are believed to play a physiological role in the stimulation and satiation of hunger [20] Angiotensin converting enzyme (ACE) is a circulating dipeptidase with a broad substrate specificity [26] Circulating angiotensin I [13], bradykinin [25] and opiate peptides [7,12] are known to be metabolised by ACE The introduction of potent, specific ACE inhibitors such as captopril (SQ 14225) and enalapril (MK 421) has allowed the examination of the role of angiotensin in drinking behaviour [8], salt appetite [10] and blood pressure regulation [1] and opiates in the sensation of pain [11] and control of respiratory rate [21]

Given this evidence that circulating peptides such as CCK, VIP or opiates modulate ingestive behaviour [20] and that circulating ACE is an important enzyme in circulating peptide metabolism [26] we have examined using the specific, potent ACE inhibitors captopril and enalapril, whether ACE plays a role in feeding and drinking behaviour following food deprivation Further, given that the pH of captopril solutions is low and might influence ingestive behaviour we have also examined the effect of both buffered and unbuffered captopril treatment on feeding and drinking behaviour

METHOD

Male Sprague Dawley rats (300–450 g) were maintained in individual cages with ad lib access to food (GR2+, Clarke King) and water except where indicated below Animals were adapted to housing and feeding conditions for 7 days prior to usage and a sham injection procedure used prior to experiments to adapt animals to experimental procedures The

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light cycle occurred from 0700–1900 hr with treatment regimes designed such that intakes in all experiments were measured between 1000 and 1400 hr

Effect of Captopril on Food Intake Following 24 Hr Food Deprivation

Thirty rats were deprived of food, but not water, for a 24 hr period commencing at 1000 hr One hour prior to the return of food animals were given an intraperitoneal injection of either vehicle (0 9% saline, pH=6 0), unbuffered captopril (50 mg/kg, pH=2 1) or buffered captopril (50 mg/kg, pH=5 7) PH was adjusted with 5 M NaOH Food intake was measured 1, 2 and 4 hr following the return of food This dose of captopril is known to effectively blockade ACE [5,27] and antagonize drinking behaviour believed to be dependent on angiotensin II [10,28]

Effect of Captopril on Food Intake Following 2-Deoxyglucose Treatment

Twenty rats were pretreated with either vehicle or unbuffered captopril 1 hr prior to administration of 2-deoxyglucose (300 mg/kg, IP) to all animals Food and water intakes were measured 1, 2 and 4 hr following 2-deoxyglucose administration

Effect of Captopril on Water Intake Following 24 Hr Water Deprivation

Thirty animals were deprived of water, but not food, for a period of 24 hr One hour prior to the return of water, animals were treated with either vehicle, unbuffered captopril or buffered captopril as above Water intake was measured in each group 30 and 60 min following the return of water

Effect of Naloxone on the Reduced Food Intake Following Unbuffered Captopril Treatment

Thirty rats were deprived of food for 24 hr commencing at 1000 hr One hour prior to the return of food, animals received either vehicle, unbuffered captopril or combined captopril and naloxone (1 mg/kg, IP) treatment This dose of naloxone has been shown to alter other forms of ingestive behaviour (see review [23])

Effect of Captopril on Blood Gases and pH

In order to determine whether buffered or unbuffered captopril alters acid-base balance, blood gases and pH were determined in thirty rats pretreated for 1 hr with either vehicle, buffered or unbuffered captopril Values were determined in a Corning 168 Blood Gas analyzer

Effect of Enalapril on Food Intake Following 24 Hr Food Deprivation

Twenty animals were deprived of food for 24 hr with continuous access to water One hour prior to the return of food, animals were treated for 1 hr with vehicle (1 M PO_4 buffer, IP, pH=7 4) or an equimolar dose of enalapril (80 mg/kg, IP) PO_4 buffer was required to increase the solubility of enalapril Food intake was measured 1, 2 and 4 hr following the return of water

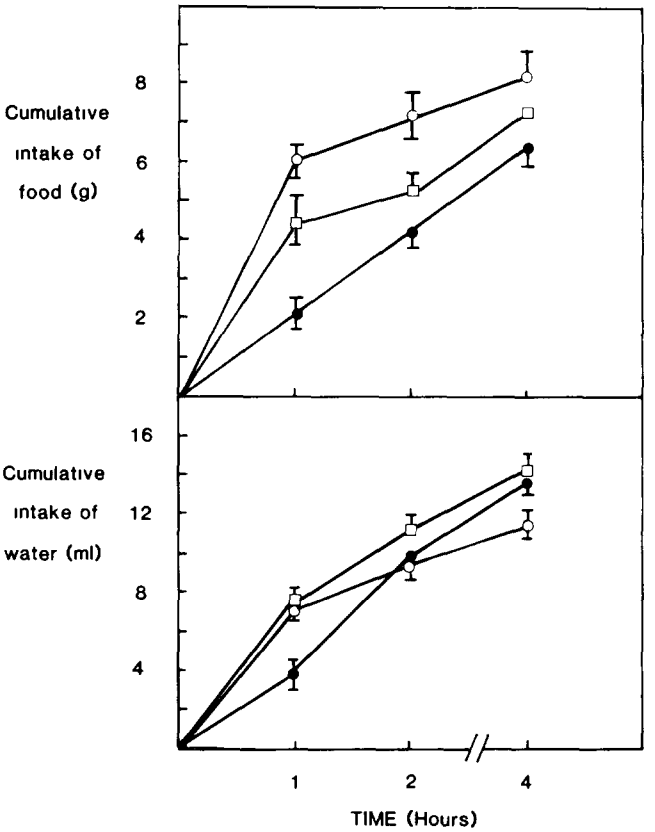

FIG 1 Cumulative food and water intake of 24 hr food deprived rats following one hour pretreatment with vehicle (○), unbuffered (●) or buffered (□) captopril Results are mean±SEM with 10 animals in each group

Statistics

Differences between treatment groups was assessed by ANOVA using a repeated measures design [3]

RESULTS

Effect of Captopril on Food Intake Following 24 Hr Food Deprivation

Unbuffered captopril significantly, $F(1,18)=20.2$, $p<0.001$, reduced the food intake of food deprived rats when compared to vehicle treated controls (Fig 1) Food intake of buffered captopril treated rats was significantly greater, $F(1,18)=7.7$, $p<0.02$, than that of unbuffered captopril treated animals The food intake of buffered captopril treated animals was still significantly lower, $F(1,18)=6.4$, $p<0.03$, than that of vehicle treated animals over the first 2 hr period (Fig 1)

Effect of Captopril on Food Intake Following 2-Deoxyglucose Treatment

Unbuffered captopril failed to significantly alter, $F(1,18)=0.1$, $p>0.9$, the food intake of rats induced by 2-deoxyglucose administration (Fig 2) The prandial water intake of buffered captopril treated rats was significantly greater, $F(1,18)=6.5$, $p<0.02$, than that of vehicle pretreated animals

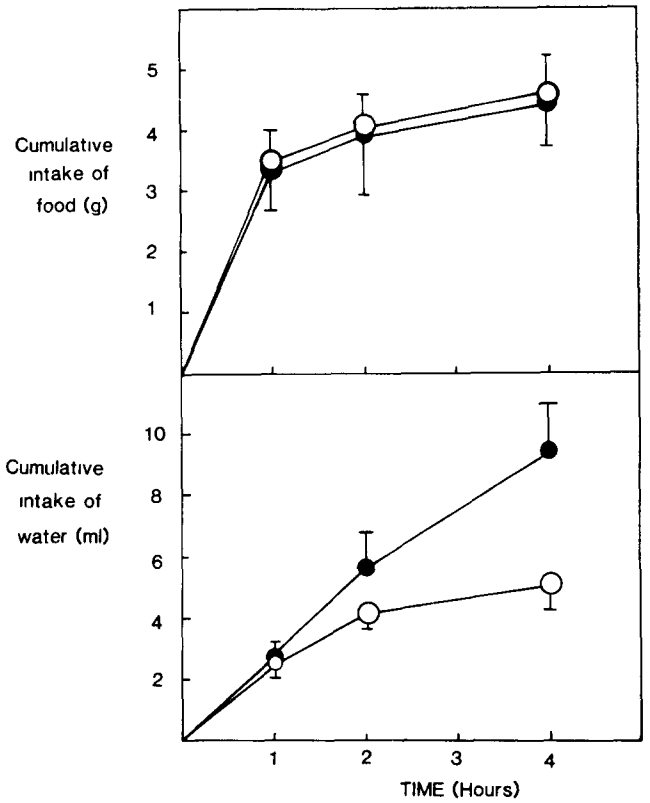

FIG 2 Cumulative food and water intake of rats receiving IP 2-deoxyglucose following one hour pretreatment with either vehicle (○) or unbuffered captopril (●) Results are mean±SEM with 10 animals in each group

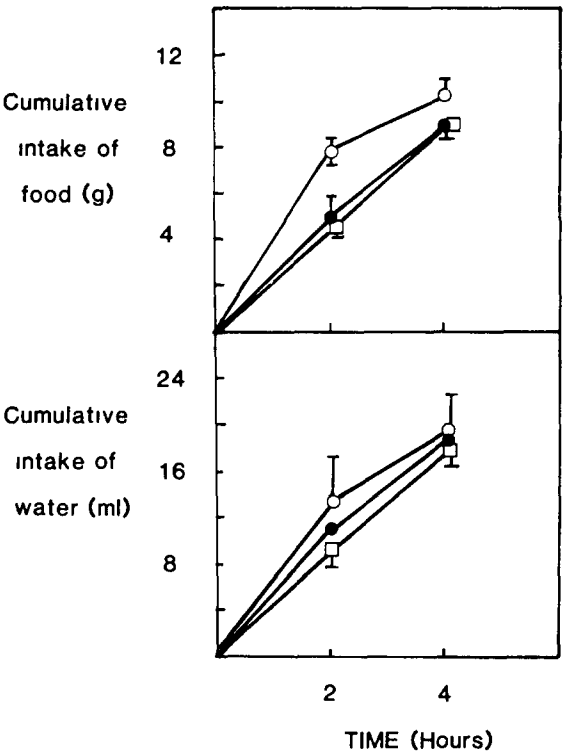

FIG 4 Cumulative food and water intake of 24 hr food deprived rats pretreated for 1 hr with either vehicle (○), unbuffered captopril (●) or concomitant captopril and naloxone (□) Results are mean±SEM with 10 animals in each group

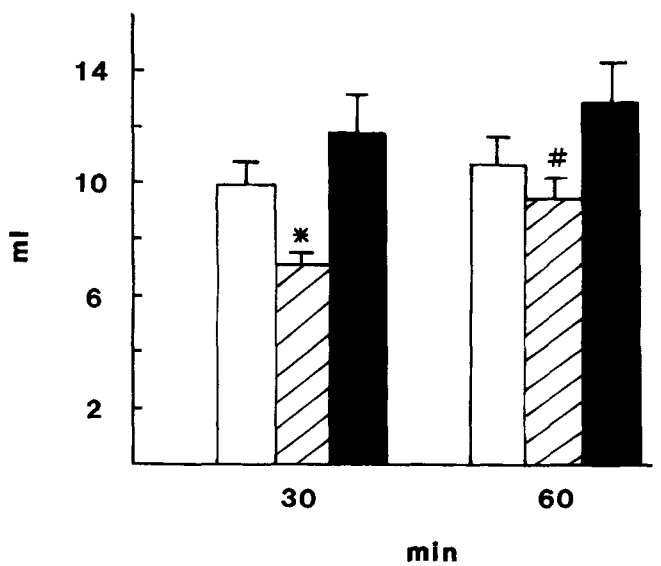

FIG 3 Cumulative water intake of 24 hr water deprived, food replete rats pretreated for 1 hr with either vehicle (0 9% saline, pH=6 0, open bars), unbuffered captopril (pH=2 1, hatched bars) or buffered captopril (pH=5 7, filled bars) prior to return of water Results are mean±SEM with 10 animals in each group * $p<0$ 05 cf vehicle, # $p<0$ 05 cf buffered captopril

Effect of Captopril on Water Intake Following 24 Hr Water Deprivation

Vehicle treated, water deprived rats drank immediately upon return of water with most drinking completed 30 min after return of water (Fig 3). Comparison of intakes in the 3 treatment groups over the one hour observation period indicated a significant treatment effect, $F(2,27)=4$ 7, $p<0.02$ Subsequent ANOVA contrasts found that animals receiving unbuffered captopril drank significantly less than either vehicle or buffered captopril treated rats, $F(1,27)=4$ 7, $p<0.04$ and $F(1,27)=12$ 6, $p<0.002$, respectively, during the first 30 min following water return (Fig 3). While there was no significant difference in water intake between vehicle and unbuffered captopril treated rats by 60 min following water return, $F(1,27)=0.6$, $p>0.4$, the intake of buffered captopril treated animals was significantly greater than that of the unbuffered captopril treated group at this time, $F(1,27)=6$ 1, $p<0$ 02.

Effect of Naloxone on the Reduced Food Intake Following Unbuffered Captopril Treatment

Naloxone failed to significantly alter, $F(1,12)=0.1$, $p>0$ 8, the reduced food intake of unbuffered captopril treated animals (Fig. 4).

Effect of Captopril on Blood Gases and pH

Both buffered and unbuffered captopril treatment failed

TABLE 1

BLOOD GASES AND pH FOLLOWING ONE HOUR PRETREATMENT WITH EITHER VEHICLE, BUFFERED OR UNBUFFERED CAPTOPRIL

Treatment	pCO_2 mmHg	pO_2 mmHg	pH
Vehicle	49 ± 2	42 ± 4	7 4 ± 0 1
Unbuffered Captopril	50 ± 2	50 ± 5	7 4 ± 0 1
Buffered Captopril	53 ± 2	49 ± 8	7 4 ± 0 1

Results are mean ± SEM with 10 animals in each group

to significantly ($p>0$ 05) alter blood gases and pH when compared to vehicle treated controls (Table 1)

Effect of Enalapril on Food Intake Following 24 Hr Food Deprivation

Enalapril failed to significantly, $F(1,18)=0$ 7, $p>0$ 4, alter the food intake of 24 hr food deprived rats when compared to vehicle treated controls (Fig 5)

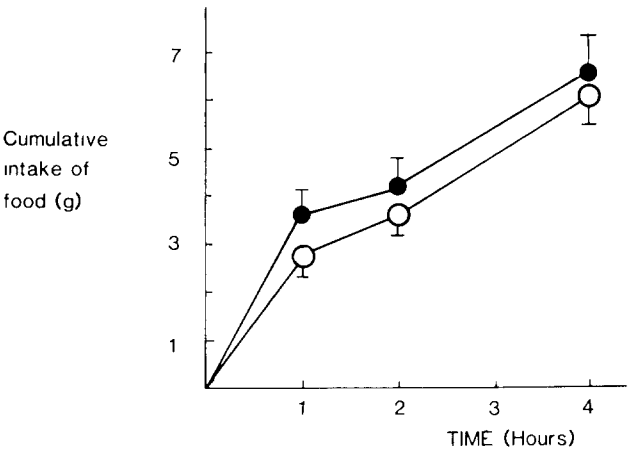

FIG 5 Cumulative food intake of 24 hr food deprived rats pretreated for 1 hr with either vehicle (○) or enalapril (●) Results are mean±SEM with 10 animals in each group

DISCUSSION

Experiments utilizing ACE inhibitors have demonstrated that endogenous peptide substrates of ACE may play a physiological role in thirst [8], salt appetite [10], blood pressure regulation [1] and renal function [14] In this study captopril reduced the food intake of food deprived rats suggesting that a peptide substrate of ACE may be involved in satiation However, captopril solutions have a low pH and may reduce ingestive behaviour by causing discomfort directly or by changes in acid-base balance However, adjustment of the pH of captopril solutions to that of the vehicle only partially reversed the action of unbuffered captopril on food intake following food deprivation suggesting that the remaining component of the reduction is due to some other action of captopril

Given that ACE blockade potentiates the action of endogenous opiate peptides [7,11] and that these peptides are believed to modulate ingestive behaviour [4, 6, 23] we examined whether naloxone altered the captopril induced reduction in food intake Naloxone, at an effective dose, failed to modify this effect of captopril. Whether kinins, angiotensin II or prostaglandins mediate this action of captopril awaits further studies

The water intake of unbuffered captopril treated rats was significantly reduced one hour following return of food when compared to buffered captopril treated rats whose intakes were not significantly different to that of vehicle treated controls This reduced prandial drinking was probably secondary to reduced food intake at this time However, this dose of captopril has been reported to reduce water intake of water deprived, food replete rats and used as evidence that angiotensin may play a role in stimulating dehydration-induced drinking [2] In order to examine whether reduction in dehydration-induced drinking in this earlier study was also pH related, we examined the water intake of 24 hr dehydrated, food replete rats given buffered and unbuffered captopril at the same dose and route of administration as this earlier study We found that, as in the earlier study, unbuffered captopril reduced water intake but that buffered captopril failed to reduce the water intake of dehydrated rats, suggesting that, as in the feeding studies noted above, it is important to consider the pH of captopril solutions when studying its effects on behaviour

Captopril failed to alter the food intake of rats following 2-deoxyglucose administration suggesting that the anorexic action of captopril depends on the model of hunger being examined This finding also suggests that hunger elicited by 2-deoxyglucose occurs independently of the site involved in the captopril induced decrease in food intake

It is now believed that the central actions of some circulating peptides are mediated by circumventricular organs of the brain such as the subfornical organ [17, 19, 22] These areas are now known to contain a high concentration of ACE [18] The doses of ACE inhibitors used in this study are those known to blockade ACE at such central sites [28] as well as in the systemic circulation [5,27] Such doses have previously been shown to reduce certain forms of thirst [2] and salt appetite [10,28] and lower blood pressure in experimental hypertension [1] probably by blocking peripheral and central ACE A one hour pretreatment period was chosen in this study as this replicates an earlier study examining the effect of captopril on ingestive behaviour which only found an effect with 45–60 min pretreatment with this drug and no action with shorter or longer pretreatment periods [2]

Enalapril, a more potent ACE inhibitor than captopril [5,27], at an equimolar dose to that of captopril, failed to alter the food intake of food deprived rats This suggests that the anorexic action of captopril may be specific to this ACE inhibitor Clinical studies have reported that captopril interferes with taste in some patients [15] Enalapril which does not possess a sulphydryl moiety does not alter taste [9] to the same extent and therefore the anorexic action of captopril may be due to the sulphydryl group it contains, a moiety which has been implicated in alterations in taste when contained in other drugs [24]

ACKNOWLEDGEMENTS

This work was supported by grants from the National Health and Medical Research Council of Australia We thank Sue Spargo and Dale Green for technical assistance

REFERENCES

1 Antonaccio, M J , B Rubin and Z P Horovitz Effects of captopril in animal models of hypertension *Clin Exp Hypertens* **2:** 613–637, 1980

2 Barney, C C , M J Katovich and M J Fregly The effect of acute administration of an angiotensin converting enzyme inhibitor, captopril (SQ 14,225), on experimentally induced thirsts in rats *J Pharmacol Exp Ther* **212:** 53–57, 1980

3 Brecht, M L and J A Woodward *Ganova A General Personal Computer Programme for Univariate and Multivariate Analysis of Variance* Dept Psychology, Univ of California, Los Angeles, 1283 Franz Hall, Los Angeles, CA, 90024

4 Brown, D R , M S Blank and S G Holtzman Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats *Life Sci* **26:** 1535–1542, 1980

5 Cohen, M L and K D Kurz Angiotensin converting enzyme inhibition in tissues from spontaneously hypertensive rats after treatment with captopril or MK421 *J Pharmacol Exp Ther* **220:** 63–69, 1982

6 Cooper, S J and D J Sanger Endorphinergic mechanisms in food, salt and water intake an overview *Appetite* **5:** 1–6, 1984

7 Di Nicolantonio, R , J S Hutchinson, Y Takata and M Veroni Captopril potentiates the vasodepressor action of met-enkephalin in the anaesthetized rat *Br J Pharmacol* **80:** 405–408, 1983

8 Di Nicolantonio, R , F A O Mendelsohn and J S Hutchinson Central angiotensin converting enzyme blockade and thirst *Pharmacol Biochem Behav* **18:** 731–735, 1983

9 Dollery, C T Safety and efficacy of Enalapril Summing up the evidence *J Hypertens* **1:** Suppl 1, 155–157, 1983

10 Elfont, R M and J T Fitzsimons The effect of captopril on sodium appetite in adrenalectomized and deoxycorticosterone-treated rats *J Physiol (Lond)* **365:** 1–12, 1985

11 Ercan, Z S , M Ilhan and R K Turker Alterations by captopril of pain reactions due to thermal stimulation of the mouse foot interactions with morphine, naloxone and aprotinin *Eur J Pharmacol* **63:** 167–177, 1980

12 Erdos, E G , A R Johnson and N T Boyden Hydrolysis of enkephalin by cultured human endothelial cells and by purified peptidyl dipeptidase *Biochem Pharmacol* **27:** 843–848, 1978

13 Giese, J , S Rasmussen, M D Nielsen and H Ibsen Biochemical monitoring of vasoactive peptides during angiotensin coverting enzyme inhibition *J Hypertens* **1:** Suppl 1, 31–36, 1983

14 Harris, P J and J P Munro Reversal by angiotensins II and III of the effects of converting enzyme inhibition on renal electrolyte excretion in rats *J Physiol (Lond)* **351:** 491–500, 1984

15 Heel, R C , R N Brogden, T M Speight and G S Avery Captopril a preliminary review of its pharmacological properties and therapeutic efficacy *Drugs* **20:** 409–452, 1980

16 Kissileff, H R and T B VanHallie Physiology of the control of food intake *Annu Rev Nutr* **2:** 371–418, 1982

17 McKinley, M J An important region for osmoregulation the anterior wall of the third ventricle *News Physiol Sci* **2:** 13–16, 1987

18 Mendelsohn, F A O , S Y Chai and M Dunbar In vitro autoradiographic localization of angiotensin-converting enzyme in rat brain using ^{125}I-labelled MK 351A *J Hypertens* **2:** Suppl 3, 41–44, 1984

19 Mendelsohn, F A O , J M Quirion, J M Saavedra, G Aguilera and K J Catt Autoradiographic localization of angiotensin II receptors in rat brain *Proc Natl Acad Sci USA* **81:** 1575–1579, 1984

20 Morley, J E , T J Bartness, B A Gosnell and A S Levine Peptidergic regulation of feeding *Int Rev Neurobiol* **27:** 207–298, 1985

21 Oktay, S , R Onur, I Mustafa and R K Turker Potentiation of the morphine-induced respiratory rate depression by captopril *Eur J Pharmacol* **70:** 257–262, 1981

22 Quirion, R , M Dalpe, A De Lean, J Gutkowska, M Cantin and J Genest Atrial natriuretic factor (ANF) binding sites in brain and related structures *Peptides* **5:** 1167–1172, 1984

23 Sanger, D J Endorphinergic mechanisms in the control of food and water intake *Appetite* **2:** 193–208, 1981

24 Schiffman, S S Taste and smell in disease *N Engl J Med* **308:** 1275–1279, 1983

25 Skeikh, I A and A P Kaplan Studies of the digestion of bradykinin, lys-bradykinin, and des-arg^9-bradykinin *Biochem Pharmacol* **35:** 1951–1956, 1986

26 Soffer, R L Angiotensin-coverting enzyme and the regulation of vasoactive peptides *Annu Rev Biochem* **45:** 73–94, 1976

27 Takata, Y , R Di Nicolantonio, F A O Mendelsohn, J S Hutchinson and A E Doyle A comparison of the activity of the angiotensin converting enzyme inhibitors SQ 14225, SA 446 and MK 421 *Clin Exp Pharmacol Physiol* **10:** 131–145, 1983

28 Weiss, M L , K E Moe and A N Epstein Interference with central actions of angiotensin II suppresses sodium appetite *Am J Physiol* **250:** R250–R259, 1986