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The angiotensin IV/AT_4 receptor

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Abstract. The angiotensin AT_4 receptor was originally defined as the specific, high-affinity binding site for the hexapeptide angiotensin IV (Ang IV). Subsequently, the peptide LVV-hemorphin 7 was also demonstrated to be a bioactive ligand of the AT_4 receptor. Central administration of Ang IV, its analogues or LVV-hemorphin 7 markedly enhance learning and memory in normal rodents and reverse memory deficits observed in animal models of amnesia. The AT_4 receptor has a broad distribution and is found in a range of tissues, including the adrenal gland, kidney, lung and heart. In the kidney Ang IV increases renal cortical blood flow and decreases Na^+ transport in isolated renal proximal tubules. The AT_4 receptor has recently been identified as the transmembrane enzyme, insulin-regulated membrane aminopeptidase (IRAP). IRAP is a type II integral membrane spanning protein belonging to the M1 family of aminopeptidases and is predominantly found in GLUT4 vesicles in insulin-responsive cells. Three hypotheses for the memory-potentiating effects of the AT_4 receptor/IRAP ligands, Ang IV and LVV-hemorphin 7, are proposed: (i) acting as potent inhibitors of IRAP, they may prolong the action of endogenous promnestic peptides; (ii) they may modulate glucose uptake by modulating trafficking of GLUT4; (iii) IRAP may act as a receptor, transducing the signal initiated by ligand binding to its C-terminal domain to the intracellular domain that interacts with several cytoplasmic proteins.

Key words. Insulin-regulated aminopeptidase; oxytocinase; memory; GLUT4.

Introduction

Since the initial description of the angiotensin AT_4 receptor in 1992 as the specific, high-affinity binding site for the hexapeptide angiotensin IV (VYIHPF, Ang IV) [1] it was suspected to be classically a G protein coupled receptor. However, we have purified the receptor and identified it as insulin-regulated aminopeptidase (IRAP) [2], an abundant protein that is found in specialised vesicles containing the insulin-sensitive glucose transporter GLUT4 [3]. In addition, the soluble form of IRAP present in maternal serum is known as oxytocinase, an enzyme which efficiently metabolises oxytocin and vasopressin [4]. Hence, studies on the physiological effects of Ang IV, acting on the 'AT_4 receptor', carried out prior to its identification now have to be re-evaluated in terms of a peptide-enzyme interaction. Investigations into the physiological effects mediated by the AT_4 receptor are further confounded by the fact that Ang IV is also a weak agonist of the angiotensin AT_1 receptor. For example, Ang IV was vasodilatatory in most vascular beds but induces vasoconstriction in rat mesenteric artery [5] and decreases renal and mesenteric blood flow [6] by binding to the AT_1 receptor.

AT_4 receptor: historical perspectives

Agonists and antagonists of the AT_4 receptor

Modifications have been made to the N-terminal residues of Ang IV to increase its half-life by preventing its degra-

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dation by aminopeptidases [7]. Structure activity studies reveal that the first three amino acid residues of Ang IV are critical for binding to the AT_4 receptor [8]. An N-terminal primary α-amine and an L-conformation for the first amino acid α-carbon are requisites for high-affinity binding of the hexapeptide to the AT_4 receptor [7]. An activated aromatic ring in the side chain of amino acid residue 2 and a hydrophobic amino acid in position 3 are important for high-affinity binding [9]. Discrete modifications to the subdomains of the valine residue in position 1, in particular, a straight-chain aliphatic moiety containing four carbons resulted in a 100-fold higher affinity analogue, Nle^1-Ang IV [7]. Replacement of the amide bond between residues 1 and 2 of Nle^1-Ang IV (norleucinal Ang IV) with a methylene peptide bond isostere [ΨCH2-NH)] results in a 10-fold reduction in affinity [7]. Both these analogues of Ang IV exhibit agonist properties of Ang IV.

An AT_4 receptor 'antagonist' was generated by the replacement of the amide bonds between Val^1 and Tyr^2 and between Val^3 and His^4 with [ΨCH2-NH)]. Divalinal Ang IV exhibited very similar affinity as Ang IV in binding to the AT_4 receptor in bovine adrenal membranes [10], although in our laboratory, divalinal has 20-fold lower affinity than Ang IV (K_i of 445 and 17 nM, respectively) in competing for binding to the human AT_4 receptor [11]. Divalinal Ang IV was found to antagonise some of the physiological actions of Ang IV on vasodilatation [10], increased urinary sodium excretion [12], enhancement of long-term potentiation in rat hippocampus [13] and facilitation of K^+-evoked acetylcholine release [14].

The isolation and identification of structurally distinct peptide ligands that bind with high affinity to the AT_4 receptor has contributed significantly to distinguishing effects mediated by the AT_4 receptor from those that are non-specific. LVV-hemorphin 7 (LVVYPWTQRF) was isolated from sheep cerebral cortex based on its ability to compete for ^{125}I-Ang IV binding with high affinity (K_i 55 nM [15]). In addition, radiolabelled LVV-hemorphin 7 binds to the same sites as ^{125}I-Ang IV [16]. This decapeptide also mimics some of the biological effects of Ang IV, including facilitation of cholinergic neurotransmission in rat hippocampal slices [14] and enhancement of spatial learning [17].

Role of AT_4 receptor in memory processing

The most significant and reproducible effect of angiotensin IV and other AT_4 receptor ligands is facilitation of learning and memory, demonstrated in a number of behavioral paradigms. In early studies, the effect of Ang IV on memory was observed when 1 nmol Ang IV was administered intracerebroventricularly 15 min prior to the first trial in aversive-conditioning paradigms. Ang IV significantly improved acquisition in condition avoidance responses [18] and enhanced retention and retrieval in the passive avoidance task [18, 19]. In the Morris water maze task, which tests spatial learning, chronic infusion of the more stable Ang IV analogue, Nle^1-Ang IV, at a dose of 0.1 or 0.5 nmol/h, facilitated acquisition on the first 2 days of the trials as measured by decreased latency and decreased path distance swum to locate the submerged platform [20]. This improved performance was no longer evident by day 3 of the acquisition trials. Interestingly, infusion of the AT_4 receptor 'antagonist', divalinal Ang IV (0.5 or 5 nmol/h) by itself, significantly impaired performance [20].

We have recently demonstrated in another spatial learning task, the Barnes circular maze, that a single acute injection of Nle^1-Ang IV into the lateral ventricle, 5 min before the first trial on the first day of testing, accelerates the ability of the animals to learn the location of the hidden escape chamber. Control animals given artificial cerebrospinal fluid (CSF) took up to 8 days to achieve learner criteria whereas animals given 100 pmol or 1 nmol Nle^1-Ang IV all learnt by day 5. Similarly, administration of 100 pmol LVV-hemorphin 7 produced the same effect as Nle^1-Ang IV in this paradigm – providing evidence that the facilitation of spatial learning is mediated by the AT_4 receptor.

Ang IV was also reported to induce improvement in long-term memory even in a species of crab, *Chasmagnathus,* when administered into the dorsal cephalothoracic-abdominal membrane at a dose of 5 pmol [21]. This group concluded that the effect of Ang IV on contextual-signal memory in crab is limited to exogeneously applied peptide, as the antagonist, divalinal by itself, does not have an amnestic effect [22].

Not only are the AT_4 ligands effective in improving learning and memory in normal rodents, these compounds can overcome memory deficits induced by scopolamine treatment [23, 24] or bilateral perforant pathway lesions [20]. In support of the memory-enhancing effects of the AT_4 ligands, both Ang IV and Nle^1-Ang IV enhance long-term potentiation in the rat dentate gyrus in vivo [13] and in the CA1 region of rat hippocampal slices in vitro [25]. Both Ang IV and LVV-hemorphin 7 facilitate K^+-evoked acetylcholine release from rat hippocampal slices [14]. There is a striking degree of overlap between the distribution of the AT_4 receptors in the brain with cholinergic neurons and their projections particularly in the septum, hippocampus and cerebral cortex [26]. This suggests that potentiation of cholinergic neurotransmission may comprise, in part, the mechanism by which AT_4 ligands enhance memory.

Role of the Ang IV/AT_4 receptor in other tissues

Ang IV, and its more stable analogues, caused vasorelaxation in cerebral [27] and renal [12] vascular beds, effects

attributed to enhanced endothelial intracellular calcium release [28] and increased endothelial nitric oxide synthase (ecNOS) activity [29]. In porcine pulmonary endothelial cells, activation of ecNOS activity was mediated by mobilization of intracellular Ca^{++} and increased expression of the endoplasmic reticulum Ca^{++}-binding protein calreticulin [30]. Ang IV also stimulated phosphotidylinositol 3 kinase and phosphotidylinositol-dependent kinase-I activity and induced phosphorylation of protein kinase B and extracellular signal-related kinase (ERK) I and II [31] in these cells. These multiple kinase pathways are thought to regulate the cellular proliferative effect of the peptide [31]. In both porcine and bovine aortic endothelial cells, Ang IV induced plasminogen activator inhibitor 1 expression, suggesting a role for Ang IV in fibrinolysis [32, 33].

The AT_4 receptor has also been characterised in the heart, where Ang IV stimulates protein synthesis in rabbit cardiac fibroblasts [34] and Nle^1-Ang IV reduces mechanically induced expression of the immediate early genes c-fos and egr-1 in the isolated rabbit heart [35]. In the same isolated preparation, Nle^1-Ang IV also reduced left ventricular pressure generating and ejection capabilities and enhanced the sensitivity of pressure development to volume change and acccelerated relaxation [36].

In the rat kidney, the distribution of the AT^4 receptor was reported to occur in high levels in the proximal tubules [37, 38], although in other species, including the rabbit and sheep, the binding site is concentrated in the glomerular tufts, in the thick ascending limbs of the loop of Henle and in the collecting duct (fig. 1) [unpublished observations]. Infusion of Ang IV into the renal artery of rats resulted in increased renal cortical blood flow and urinary sodium excretion [12]. In isolated rat proximal tubules Ang IV inhibits sodium transport [38], and in the human proximal tubular HK2 cells, Ang IV dose-dependently enhances ERKII and p38 kinase phosphorylation [39] and PAI1 messenger RNA (mRNA) expression [40]. In the porcine proximal tubular LLC-PK_1/Cl_4 cells, Ang IV causes a rapid phosphorylation of p125-focal adhesion kinase and p-68 paxillin [41], suggesting a direct effect on these signalling molecules.

The AT_4 receptor is found in high concentrations in the adrenal gland, and the biochemical properties of the bovine adrenal receptor is well-characterized [42, 43]. The AT_4 receptor has also been detected in other tissues, including spleen, colon, prostate and bladder [44]; however, its role in these tissues has not been reported.

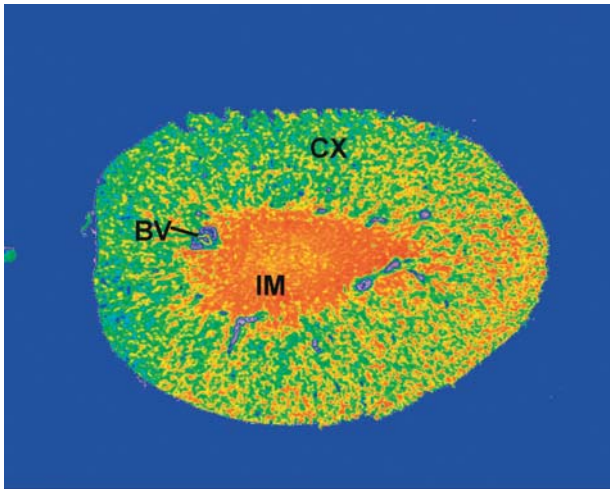

Figure 1. Pseudocolour image of the distribution of AT_4 receptor/IRAP in the kidney of the *Macaca fascicularis* as revealed by specific ^{125}I-[Nle^1] Ang IV binding. The colour code is as follows: red, very high levels of binding; orange and yellow, high; green, moderate; blue, low to undetectable levels of binding. Note the abundance of AT_4 receptor/IRAP in the inner medulla (IM). In the cortex (CX), AT_4 receptor/IRAP is associated with the distal and proximal tubules. Moderate levels of AT_4 receptor/IRAP occur in the blood vessels (BV).

The AT_4 receptor is insulin-regulated aminopeptidase or oxytocinase: re-evaluation of the field

IRAP

We purified the AT_4 receptor from bovine adrenal glands, an abundant source of the receptor. The receptor was cross-linked to a photoactivatable analogue of Ang IV, [^{125}I]Nle^1-$BzPhe^6$-Gly^7-Ang IV, solubilised and purified by anion-exchange chromatography and gel electrophoresis. The protein that co-migrated with the radioactive band was excised, and the peptide sequence was obtained from a tryptic peptide by tandem mass spectrometry. This procedure identified the AT_4 receptor as insulin-regulated aminopeptidase (IRAP) [2].

IRAP was initially purified and cloned in 1995 by Keller et al. as a marker protein for a specialised class of vesicles containing the insulin-responsive glucose transporter GLUT4, which is present in adipose and skeletal muscle cells [3]. IRAP accompanies GLUT4 in these insulin-responsive tissues, cycling within intracellular compartments in the basal state or translocating with GLUT4 to the plasma membrane in response to insulin [45]. Disruption of IRAP expression in IRAP–/– mice results in a 40–85% decrease in GLUT4 levels in skeletal muscles and fat cells with an accompanying decrease in both basal and insulin-stimulated glucose uptake [46]. Despite the close association of IRAP with GLUT4, its role in insulin-responsive tissues is not clearly defined, although IRAP is known to play a role in the trafficking of GLUT4 vesicles [47], thereby regulating glucose uptake into these cells [47]. In support of this role is the presence of two dileucine motifs with surrounding acidic clusters in the N-terminal tail of IRAP which are important for intracellular trafficking and sorting [47, 48]. Within GLUT4 vesicles, IRAP is oriented such that its 109-

amino acid N-terminal tail projects into the cytoplasm [3], enabling its tethering to intracellular structures. Injection of the N-terminal tail (GST-IRAP1-109) into 3T3-LI cells results in the translocation of GLUT4 vesicles to the plasma membrane [47]. However, although the IRAP–/– mice exhibit impaired glucose uptake in muscle and adipose cells, they maintain normal glucose homeostasis – fed and fasted blood glucose and insulin levels were indistinguishable from wild-type controls [46].

The cytopalsmic domain of IRAP interacts with three distinct classes of proteins – tankyrase [49], acyl-coenzyme A (CoA) dehydrogenase ACDs [50] and the formin protein homologue FHOS [51]. The interaction of these proteins with IRAP is thought to be important for tethering GLUT4 vesicles to intracellular compartments or the trafficking of the vesicles to the plasma membrane. Tankyrase contains a PARP domain [which catalyzes poly (ADP-ribosyl)ation] and an ANK domain (which binds telomere repeat binding factor-1, IRAP and a 185-kDa tankyrase binding protein) [52]. Although tankyrase is co-localized with GLUT4 in the trans-Golgi network and is a substrate of MAP kinase which is phosphorylated upon insulin stimulation [49], the interaction between IRAP and tankyrase is not disrupted by this insulin-induced phosphorylation and therefore not likely to be involved in trafficking of GLUT4 vesicles. The other two interacting proteins are more likely candidates. Administration of inhibitors of ACDs (enzymes which participate in the β-oxidation of fatty acids in mitochondria) into 3T3-L1 adipocytes resulted in translocation of GLUT4 vesicles to the plasma membrane and enhanced glucose uptake into the cells [50]. Moreover, these inhibitors block the association of the proteins with IRAP. Overexpression of FHOS or its splice variant, FHOS78, in the L6 skeletal muscle cell line enhances basal and insulin-induced glucose uptake [51]. It was postulated that FHOS, through its interaction with profillin IIa, may play a role in tethering GLUT4 vesicles to the cytoskeleton.

Brain IRAP: role in glucose uptake?

In view of the striking effects of AT_4 ligands in enhancing memory in normal rodents and in reversing the memory deficits in animal models of amnesia, it is important to determine if AT_4 ligands affect glucose uptake. Cognitive function correlates with enhanced glucose utilisation, particularly in the hippocampus – the more complex the task, the greater the glucose uptake [53]. The predominant forms of glucose transporter found in the brain are (i) GLUT1, which is found in endothelial cells and glial processes and is thought to be responsible for facilitating glucose uptake from the bloodstream into the brain [54], (ii) GLUT3, which is constitutively expressed on the plasma membrane of neurons [55] and (iii) GLUT5, which is found in microglia. The insulin-responsive glucose transporter GLUT4 has also been detected in neurons in the brain [56–58], and we have recently demonstrated that in some regions of the brain, particularly in the hippocampus, GLUT4 is found in neurons that also express IRAP (fig. 2). There is a high degree of overlap between the distribution of IRAP and GLUT4 within the same intracellular compartments in these neurons (fig. 2). This suggests the presence of an analogous GLUT4 vesicle population in neurons which in the basal state recycle within intracellular compartments and when stimulated translocate to the plasma membrane to facilitate glucose uptake into neurons.

Role of glucose in memory processing

Exogenous glucose, administered either peripherally or centrally, enhances cognitive function in animals and in humans. Intraperitoneal injection of glucose enhances memory performance in rats in the radial arm maze and reverses scopolamine-induced memory deficits [59], enhances retention in the passive avoidance task [60] and facilitates memory retention of a habituation response in mice [61]. Glucose also alleviates memory impairment induced by scopolamine in pigeons [62]. In humans, oral glucose administration enhances performance of complex cognitive tasks in healthy young adults, for example in a word recognition task [63] and in the recall of narrative prose passages [64]. The effect of glucose on cognitive function is more pronounced in older subjects [65]. The facilitative effect of exogenous glucose on cognitive performance may be the result of increased extracellular glucose in the brain available for uptake as an additional energy source for processing complex cognitive tasks. In addition, glucose facilitates acetylcholine release during the performance of a spatial task [66] or spontaneous alternation task [67].

In view of the effects of glucose in facilitating memory and enhancing cognitive function, one possible mechanism of action of AT_4 ligands could be the facilitation of glucose uptake into neurons. We propose that IRAP together with GLUT4 is translocated to the cell surface when neurons in the hippocampus are activated during performance of memory paradigms. The binding of AT_4 ligands to IRAP may result in the sustained presence of both IRAP and GLUT4 at the cell surface, facilitating the uptake of more glucose into the cell.

Aminopeptidase activity of IRAP

IRAP, EC 3.4.11.3, belongs to the M1 family of zinc-dependent metallo-peptidases, possessing two characteristic motifs in its large C-terminal domain, which is extracellular when the protein is at the plasma membrane: the highly conserved zinc-binding motif, HEXXH-X-E, and a GXMEN motif that is believed to be important for exo-

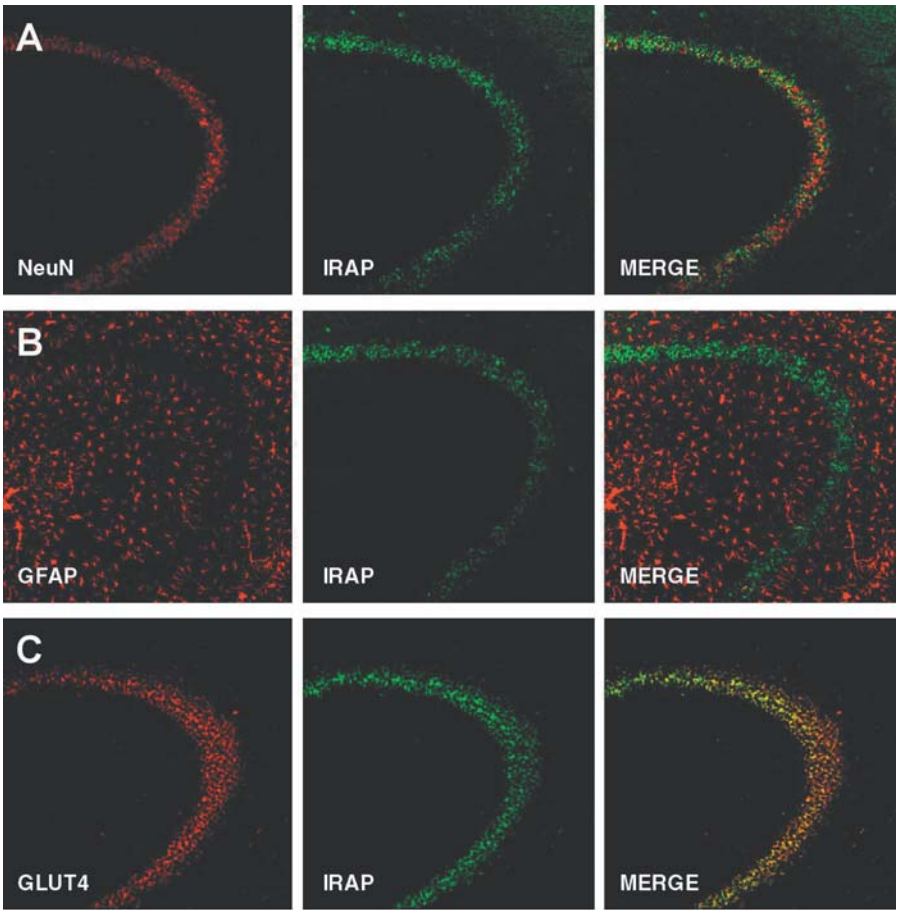

Figure 2. Dual-label immunohistochemistry illustrating the co-localisation of IRAP and GLUT4 immunoreactivity in pyramidal neurons of the rat hippocampus visualised by scanning confocal microscopy. The localisation of IRAP (green) is compared to that of (*A*) the neuronal nuclear marker, NeuN (red); (*B*) the glial marker, GFAP (red); and (*C*) insulin-regulated glucose transporter, GLUT4 (red). Merged images illustrate that IRAP immunoreactivity is found in the hippocampal cell layer which expresses NeuN but does not overlap with the expression of GFAP, indicating a neuronal localisation of IRAP. Further, the distribution of IRAP coincides almost completely with that of GLUT4, suggesting co-localisation at a subcellular level.

peptidase activity [4]. The soluble form of IRAP is known as placental leucine aminopeptidase (P-LAP) or oxytocinase [68, 69], and was characterised and subsequently cloned from a human placental library as a major enzyme present in maternal serum responsible for the breakdown of oxytocin [4]. IRAP is also capable of hydrolysing vasopressin, lys-bradykinin, angiotensin III, met-enkephalin, dynorphin A 1–8, neurokinin A, neuromedin B, somatostatin and cholecystokinin 8 [11, 70–72].

AT_4 ligands are inhibitors of IRAP

Both Ang IV and LVV-hemorphin 7 are relatively high affinity competitive inhibitors of IRAP [11]. These two peptides are not cleaved to any appreciable extent by cells transfected with human IRAP and are therefore not substrates of the enzyme [11]. We propose that the AT_4 ligands mediate their physiological effects by binding to the active site of IRAP, inhibiting its catalytic activity [73]. In view of the fact that IRAP cleaves vasopressin, oxytocin and met-enkephalin efficiently [11], it is likely that the AT_4 ligands, by binding to IRAP, may serve to prolong the half-life of one or more of these neuropeptide substrates. Amongst the peptides that are cleaved by IRAP, vasopressin, oxytocin, cholecystokinin-8 (CCK-8) and somatostatin have all been demonstrated to facilitate learning and memory.

IRAP substrates with memory-enhancing properties

The effect of vasopressin on facilitating memory consolidation and retrieval is well-established in the passive avoidance paradigm [74–76]. Vasopressin has also enhances performance in the Go-No Go visual discrimination task in mice [77], although its effects in spatial learning and food reward tasks are not as convincing [75, 76, 78]. Vasopressin, or its derivative NC1900, also reverse

memory deficits induced by scopolamine [79], transient forebrain ischemia [80] and vasopressin deficiency [75]. Interestingly, recent studies suggest that N-terminal truncated fragments of vasopressin, products of vassopressin degradation by IRAP [81], are just as effective in some memory paradigms [74, 79, 82–84]. Although oxytocin was initially shown to have opposing effects to vasopressin in some of the behavioural paradigms [74, 76], a recent study reveals that oxytocin improves reference memory (a long-term form of memory), but not working memory (short-term memory), in female mice [85], an effect which correlates with increased CAmP response element binding protein (CREB) phosphorylation and induction of long-term potentiation in hippocampal slices [85].

CCK-8, or CCK B receptor agonists, have memory-enhancing effects [86, 87], including facilitating memory consolidation in the inhibitory avoidance paradigm [88] and the two-trial memory task [89] and reversing performance deficits in aged Fischer rats [90] and spatial recognition impairment induced by stress [91]. Although somatostatin is not generally thought to be associated with cognitive function, somatostatin 14, which is a substrate of IRAP, also facilitates acquisition in spatial tasks [92], and depletion of endogenous somatostatin with cysteamine results in memory deficits that are reversed by somatostatin treatment [93, 94]. It is therefore possible that AT_4 ligands, by binding to IRAP, may prolong the half-life of any of these neuropeptides, particularly in brain regions that are involved in cognitive processing.

Inhibitors of prolyl endopeptidase, another peptidase that breaks down vasopressin and oxytocin, also has memory-enhancing properties, reversing memory deficits after cerebral ischemia [95, 96], bilateral hippocampal lesion [97] or ablation of the nucleus basalis magnocellularis [98]. These compounds also ameliorate age-related memory impairment in rats [99] and mice [100]. The effects are thought to be mediated by reversing central cholinergic dysfunction [99, 101] or by prolonging the half-life of promnesic neuropeptides, including vasopressin, substance P, thyrotropin-releasing hormone [101, 102] and α-melanocyte-stimulating hormone [103].

Effect of the 'AT_4 receptor antagonist' divalinal Ang IV

Interestingly, the 'AT_4 receptor antagonist' divalinal Ang IV is also a competitive inhibitor of IRAP, albeit at much lower affinity [11]. In contrast to Ang IV, chronic infusions of divalinal Ang IV have a detrimental effect on performance in the Morris water maze [20]. Pretreatment with divalinal Ang IV blocks the effect of Ang IV on enhancement of normal long-term potentiation in rat dentate gyrus in vivo [13]. Co-administration of divalinal Ang IV with Ang IV or LVV-hemorphin 7 attenuates the effects of the peptides on potassium-evoked acetylcholine release from rat hippocampal slices [14]. The antagonistic effect of divalinal Ang IV may be due to the higher doses of the compound used, since at lower concentrations, divalinal Ang IV can mediate the same effects as Ang IV [39, 104]. In addition, many of the physiological effects exerted by Ang IV and LVV-hemorphin 7 exhibit biphasic dose-response relationships, whereby at high concentrations their effect is lost [39, 104, 105].

IRAP: role in signalling?

IRAP is the only member of the M1 family of aminopeptidase with a large (109-amino acid) N-terminal tail. At the plasma membrane, the catalytic domain is extracellular and the amino-terminal domain is cytoplasmic, whereas in GLUT4 vesicles, the amino terminus projects into the cytoplasm and the catalytic domain is present within the lumen. In addition to the dileucine motifs and acidic clusters present in this cytoplasmic domain, there are also potential Tyr phosphorylation sites in the vicinity of the acidic clusters as well as several Ser and Thr residues. IRAP is phosphorylated by protein kinase c-ζ at Ser^{80} and Ser^{91}, but the effect of phosphorylation of the protein on cellular trafficking and signalling is not known. Ang IV and its analogues induce phosphorylation of ERKII and p38 kinase [39] and p125-focal adhesion kinase and p-68 paxillin [41] in kidney cell lines in a time- and concentration-dependent manner. It is difficult to reconcile some of the earlier observations on Ang IV-induced signalling events with the effect of the peptide in inhibiting the catalytic activity of IRAP, in particular, the Ang IV-induced phosphorylation of focal adhesion kinase and paxillin, which is detected within 2 min of addition of the peptide [41]. There is therefore the novel possibility that the binding of AT_4 ligands to IRAP may result in the association with, and activation of, signalling molecules.

Conclusion

The surprising finding that the AT_4 receptor is an aminopeptidase adds a dimension of complexity to the evaluation of the physiological effects of the AT_4 ligands, in particular, the interpretation of the signalling events reported for these ligands. Since all AT_4 ligands investigated to date are inhibitors of IRAP, we postulate that one of the mechanisms by which these ligands exert their effect is by prolonging the half-life of peptide substrates of IRAP. Alternatively, the close association of IRAP with the inducible glucose transporter GLUT4 in insulin-responsive tissues suggests that AT_4 ligands may play a role

in modulating glucose uptake into cells. The distribution of IRAP within intracellular compartments in the cell under basal conditions restricts access of the AT_4 ligands to the protein. Therefore, another important consideration is the elucidation of stimuli which translocate the protein to cell surface. In view of the significant effects of AT_4 ligands in facilitating learning and memory, IRAP may represent a novel target for the development of memory-enhancing drugs.

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