quantities to be therapeutically beneficial. Thus, this experimental approach may be misleading, as trace doses do not assess the therapeutic capacity of a particular RMT pathway.

Transferrin, insulin, Apo-proteins, IGF-1, and leptin, are among an ever-increasing list of proteins that have been proposed to undergo RMT at the BBB. The transferrin/transferrin receptor (TfR) system, which mediates cellular uptake of iron, has been of particular interest as a pathway to increase uptake of biologics into the brain. Early studies with anti-TfR antibodies showed promise for the TfR pathway (Friden *et al*, 1991). Nevertheless, subsequent studies questioned how effective the TfR pathway is in driving CNS uptake of Tf itself (Crowe and Morgan, 1992), and also questioned the ability of anti-TfR antibodies to traverse the BBB and distribute throughout the brain (Moos and Morgan 2001). These, and subsequent studies, showed that anti-TfR antibodies largely remained trapped in the BBB vasculature and cast doubt on the TfR pathway as a route to transport therapeutic antibodies into the brain.

An additional limitation to understanding antibody uptake in brain has been the lack of robust and acute readouts of antibody activity. Pharmacodynamic measures of antibody function allow for the establishment of a relationship between drug levels and drug activity, termed the pharmacokinetic/pharmacodynamic (PKPD) relationship. We recently developed an antibody that would allow us to address the PKPD relationship in brain, by targeting the enzyme β-secretase (*BACE1*), an Alzheimer's disease drug target, which is required for the production of β-amyloid (Abeta; Atwal *et al*, 2011). Using this antibody, we were able to show a direct relationship between drug levels and activity (Abeta reduction) in brain. Furthermore, it was confirmed that normal antibody uptake in brain is both limited and dose-dependent, with the steady state concentrations in brain ranging from 0.05–0.2% of injected dose.

In search of a solution to increase the penetration of anti-BACE1 in brain, we turned to the most studied RMT pathway, TfR, and generated antibodies to evaluate uptake in brain in both trace and therapeutic dosing paradigms (Yu *et al*, 2011). Initial studies with our high-affinity anti-TfR antibody matched those of others; despite a substantial increase in initial drug levels as measured by trace dosing, therapeutic dosing resulted in limited uptake and was almost completely localized to the BBB vasculature. To solve this problem, we engineered anti-TfR antibodies with lower affinity for TfR, and observed an inverse relationship: reduced uptake in trace dosing paradigms and increased uptake in therapeutic dosing paradigms. More importantly, the engineered low-affinity anti-TfR antibodies distributed broadly throughout the brain, allowing us to combine anti-TfR and anti-BACE1 as a bispecific antibody to improve penetration and activity in brain. We therefore propose that RMT is indeed a viable route to the brain; however, translating these findings to humans through testing bispecifics in higher species, including safety studies, is an important next step for the anti-TfR/BACE1 program, and this approach in general.

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DISCLOSURE

The authors are paid employees of Genentech.

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Neuropsychopharmacology Reviews (2012) **37**, 302–303; doi:10.1038/npp.2011.196

Endogenous Opioids as Physiological Antidepressants: Complementary Role of Delta Receptors and Dopamine

Major depressive disorder was shown to be associated with a reduction in response to rewarding stimuli in the dopaminergic mesolimbic pathway in a recent neuroimaging study (Forbes *et al*, 2009). This neuronal network is modulated by opioids at the level of dopamine (DA) neurons and afferent structures, typically by activation of mu- and delta-opioid receptors (MORs and DORs, respectively) enhancing reward- and motivation-related processes. Therefore, a deficit in endogenous opioids, mainly enkephalins (ENKs), in the nucleus accumbens and ventral tegmental area, may lead to a decrease in the neurobiological control of mood states and reward. To develop fast-acting therapeutics for severe depression, particularly in adolescents, MORs and DORs have been investigated for potential antidepressant activity, using: (i) exogenous agonists and antagonists for both receptors and (ii) ENKs protected from their inactivating enzymes by dual inhibitors such as RB101 (N-(R,S)-2-benzyl-3-((S)(2-amino-4-methyl-thio)-butyl-dithio)-1-oxopropyl)-1-phenylalanine benzyl ester) (Noble and Roques, 2007), which induced a synaptic

enhancement of phasically released ENKs. In animal models, exogenous delta agonists such as SNC80 ((+)-4-(aR)-a-((2S,5R)-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl)-(3-methoxyphenyl)methyl)-*N,N*-diethylbenzamide induce highly significant antidepressant effects whereas mu agonists do not. This is associated with an increase in brain-derived neurotrophic factor mRNA (Jutkiewicz, 2006), which has been suggested to have a role in antidepressant effects, as well as an indirect increase in DA efflux into the striatum (Bosse *et al*, 2008). As frequently observed with some delta agonists, SNC80 causes seizures. This is not observed with RB101, which is effective in almost all animal models of depression, eg, decreases conditioned suppression of mobility in mice, immobility in the forced swim test in rats (Jutkiewicz, 2006) and in mice (Nieto *et al*, 2005) and escape failures in the learned helplessness test (Noble and Roques, 2007). The effects of RB101 are observed after a single administration and no tolerance is observed during chronic use (Cordonnier *et al*, 2005). Moreover, stimulation of opioid receptors by RB101 protected ENKs does not induce the undesirable side effects of morphine (Noble and Roques, 2007). The selective DOR antagonist naltrindole or DA antagonists reverse antidepressant effects of RB101 and SNC80, demonstrating that the effects are mediated by DORs, and involve the DA-dependent mesolimbic pathway. Consistently, DOR KO mice present depressive-like behaviors, which are reversed by antidepressant drugs, whereas MOR KO mice are unaffected (Filliol *et al*, 2000; Nieto *et al*, 2005). The key role of interconnected endogenous opioid and DAergic systems in mood control is demonstrated by the facilitation of antidepressant-like effects of RB101 after deafferentation of the DAergic mesolimbic pathway (Cordonnier *et al*, 2005), which increases the concentrations of PENK and ENKs. Taken together, these results suggest a phasic control of the DAergic mesolimbic pathway by ENKs, which may be impaired in depressive-like syndromes, and clinical studies are starting to investigate this endogenous opioid-DA interaction in human depression (Kennedy *et al*, 2006; Scott *et al*, 2008). The development of (i) dual orally active ENK inhibitors with strong analgesic properties and immediate antidepressant effects (Noble and Roques, 2007) and (ii) delta agonists devoid of side effects may lead to significant improvements in the treatment of depression and mood disorders.

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DISCLOSURE

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Pr Roques is financially supported as Chief Scientific Officer of Pharmaleads SAS, France.

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Neuropsychopharmacology Reviews (2012) **37**, 303–304; doi:10.1038/npp.2011.244

Oxytocin as a Potential Therapeutic Target for Schizophrenia and Other Neuropsychiatric Conditions

Compelling preclinical evidence indicates that the nonapeptide hormone oxytocin has a critical role in the regulation of brain-mediated processes that are strongly relevant to many neuropsychiatric disorders. The fact that oxytocin has long been approved for non-CNS uses in humans has provided an unusually auspicious opportunity to conduct investigations of its CNS effects in human subjects without requiring the lengthy and expensive preclinical and clinical toxicology studies that typically hinder translational human research of promising novel compounds. Taking advantage of this favorable situation, investigators have generated a plethora of small studies demonstrating that even a single dose of intranasally delivered oxytocin can have striking effects on human cognition and behavior. Though an oversimplification, these effects can broadly be characterized as pro-social in nature (review Macdonald and Macdonald, 2010). Understandably, those findings have generated much discussion about the possibility of translating oxytocin's effects into therapeutic applications, with autism spectrum disorders and social phobia garnering the most attention. However, it is schizophrenia, in which the application of oxytocin as putative therapeutic has advanced the furthest to date. Several positive findings with oxytocin in animal models with predictive validity for antipsychotics (eg Feifel and Reza, 1999; Lee *et al*, 2005) inspired our group to conduct a proof-of-concept human trial: a double-