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# Blockade of G Protein-coupled Receptors and the Dopamine Transporter By A Transmembrane Domain Peptide: Novel Strategy For Functional Inhibition Of Membrane Proteins *In Vivo**

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# Running Title: TM peptides inhibit receptors *in vivo*

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**Abbreviations:** GPCR, G protein-coupled receptor; TM, transmembrane; DAT, dopamine transporter; CD, Circular dichroism

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## Abstract

G protein-coupled receptors have a core consisting of seven transmembrane α-helices that is important in maintaining the structure of the receptor. We postulated that disruption of the transmembrane core may interfere with receptor function. In this study, the function of integral membrane proteins was disrupted *in vivo* using peptides mimicking their transmembrane domains. A peptide derived from transmembrane 7 of the D2 dopamine receptor injected unilaterally into caudate nucleus of rats challenged with apomorphine resulted in rotational behavior, indicating D2 receptor blockade. No rotational behavior was seen with a similar peptide based on the β2 adrenergic receptor and the D2 transmembrane peptide did not affect the D1 dopamine receptor, indicating that the D2 receptor-derived peptide had a specific effect. The intravenous administration of a transmembrane peptide derived from the $\alpha_1$-adrenergic receptor resulted in lowered arterial blood pressure and injection of a $\beta_1$-adrenergic receptor peptide resulted in decreased heart rate. Injection of a V2 vasopressin receptor-derived transmembrane peptide resulted in increased urine output suggesting antagonism of the effects of vasopressin. Finally, dopamine release in rat brain following cocaine administration was blocked by a transmembrane peptide based on the dopamine transporter. Circular dichroism spectroscopy of the peptides revealed α-helical structure similar to that of native transmembrane domains. Thus, TM peptides can disrupt membrane proteins *in vivo* likely by competing with native transmembrane domains. The disruption of the hydrophobic core architecture of membrane proteins represents a novel mechanism of achieving functional inhibition which may be possible to exploit in developing novel therapeutics.

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G protein coupled receptors (GPCRs) are predicted to have a tightly packed transmembrane (TM) core, consisting of seven membrane-spanning α-helical domains, which were shown recently by the crystal structure of the GPCR rhodopsin to have a highly organized structure (Palczewski et al., 2000). These helices are important in maintaining the three-dimensional structure and the pocket for agonist binding (Baldwin, 1993), necessitating the close proximity of certain conserved amino acids within neighbouring TM domains (Suryanarayana et al., 1992; Sealfon et al., 1995; Scheer et al., 1996; Perlman et al., 1997; Sheikh et al., 1999; Palczewski et al., 2000; Ballesteros et al., 2001). Molecular recognition between adjacent membrane-spanning regions of the protein occurs (Suryanarayana et al., 1992; Shai, 1995; Sheikh et al., 1999) and van der Waals interactions alone can mediate stable and specific associations between TM helices (MacKenzie et al., 1997).

The dopamine transporter (DAT), like all catecholamine transporters, belongs to a large family of plasma membrane transporters that also includes carriers for GABA, glycine, proline, taurine, and betaine (Torres et al., 2003). Although no X-ray crystallographic or high-resolution structural information is available for the topological assignments of these transporters, membrane neurotransmitter transporters have been shown to contain twelve TM domains with intracellular amino and carboxyl termini (Chen and Reith, 2000). Like the GPCRs and as expected for a multiple membrane-spanning helix protein, the relative alignment of TM domains is believed to be specific and is critical for the overall three dimensional structure of the transporter and therefore its proper function (Nelson, 1998).

Peptides as protein or protein segment mimetics are frequently used in attempts to emulate known peptide / protein ligands (Kieber-Emmons et al., 1997) such as in the case of somatostatin (Freda, 2002) and gonadotropin-releasing hormone analogues (Kiesel et al., 2002).

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However, peptides mimicking proteins or protein segments have also been extensively and successfully used as “decoys” that interfere with or imitate the normal interactions of the protein that is being mimicked (Okamoto et al., 1991; Pan et al., 1997; Tanaka et al., 1998; da Silva Tatley et al., 2003). We have shown that *in vitro* incubation of a peptide derived from TM 6 of the D1 dopamine receptor with membranes from cells heterologously expressing the D1 dopamine receptor resulted in disrupted receptor function (George et al., 1998). The D1-TM6 peptide became inserted into the hydrophobic core of the D1 receptor and disrupted the ligand binding site, leading to attenuated ligand binding and signal transduction. Similarly, the use of TM peptides *in vitro* has been shown to impair β2-adrenergic (Hebert et al., 1996) and CCR5 (Tarasova et al., 1999) chemokine receptor functions. Therefore, the inter-helical associations of GPCRs appear to be disturbed by exogenous peptides containing a TM sequence derived from the receptor itself as these peptides compete for the sites of interaction of the native TM domains, leading to a disruption of the receptor structure.

In this investigation, we examined whether the administration of TM peptides *in vivo* could attenuate receptor-mediated signal transduction and thereby elicit a behavioural or physiological response. Taking advantage of several well studied-physiological functions known to be linked directly to the function of specific GPCRs, we examined TM peptides derived from the D2 dopamine, $\alpha_1$-adrenergic, $\beta_1$-adrenergic, and V2 vasopressin receptors. In each case, the TM peptide disrupted the function of the receptor from which it was derived suggesting that these peptides may represent a novel type of antagonist. We also attempted to determine if this strategy for receptor disruption could be applied to another multiple membrane-spanning helix protein, the dopamine transporter (DAT). When a peptide derived from a TM domain of the DAT was administered to disrupt transporter function, blockade of cocaine-induced dopamine

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release was observed. Given the degree of specificity involved with interactions between TM domains in an integral membrane protein, our study indicates that there is great potential for the use of TM peptides or similar disrupters of hydrophobic core architecture as therapeutic agents.

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## Methods

**Peptides.** Peptides were synthesized using standard solid state methodology by Quality Control Biochemicals, Hopkinton, MA (unless indicated otherwise). For experiments, 5 mg of peptide was dissolved in 100 µl DMSO and 100 µl of 5% (w/v) digitonin and taken up to 1 ml with buffer consisting of 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), and 2 mM EDTA. Peptide amino acid sequences and the human receptor / transporter and TM region from which they were derived were as follows:

D2 dopamine receptor TM 7: LYSAFTWLGYVNSAVNPIIY-NH2 (sythesized by Chiron Mimotopes, San Diego CA ); D2 dopamine receptor TM 5: PAFVVYSSIVSFYVPFIVTL-NH2; V2 vasopressin receptor TM 7: LMLLASLNSCTNPWIY-NH2; dopamine transporter TM 12: ALGWIIATS-NH2; $GABA_A$ receptor subunit α TM 1: GIFNLVYW-NH2; $\alpha 1_A$ adrenergic receptor TM 7: GVFKVIFWLGYFNSCVNPL-NH2; β1 adernergic receptor TM 7: LFVFFNWLGYANSAFNPIIY-NH2; $\beta_2$ adrenergic receptor TM 6: GIIMGTFTLCWLPFFIVNI VH-COOH

**Circular dichroism spectroscopy of TM peptides**. Circular dichroism spectroscopy was performed using a JASCO 720 spectropolarimeter equipped with a Neslab variable temperature control unit. Cell pathlength = 0.1 cm; scan speed = 50 nm/min; resolution = 0.2 nm; bandwidth = 1.0 nm. Peptides were dissolved as described above to a concentration and then diluted in 50 mM SDS, 0-100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 2 mM EDTA buffer to a concntration of 40 µM. Four scans were accumulated for each run; the spectra shown represent the smoothed mean of three separate trials for each experiment. Spectra at 37°C were recorded 2 minutes after this temperature was reached.



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**D2 dopamine receptor expression and radioligand binding.** The D2 dopamine receptor was expressed in Sf9 cells, and receptor binding was carried out on membranes as described previously (Ng et al., 1994), using [$^{3}$H]spiperone 400 pM with (+)butaclamol 1μM to define specific binding.

**Photoaffinity labelling following peptide treatment.** Membranes prepared from D2 receptor-expressing Sf9 cells were incubated for 30 minutes at 37°C with 1 μg/ml TM 7 peptide or peptide vehicle. Following peptide treatment, the membranes were washed with PBS and incubated for 1.5 hours at room temperature with the photoaffinity label [$^{125}$I]4-azido-5-iodo-nemonapride (1 nM). This procedure and the synthesis of the photoaffinity compound have been described previously (Lee et al., 2000). Specific binding was defined by co-incubation with 1 μM (+)-butaclamol. The possibility that D2-TM7 peptide may be labelled with [$^{125}$I]4-azido-5-iodo-nemonapride was excluded by incubating the photoaffinity agent with the peptide alone and subjecting the mixture to a high percentage acrylamide gel electrophoresis. No crosslinking of the photoaffinity agent to the peptide was detected (data not shown).

**Animal welfare.** All procedures involving animal subjects strictly adhered to guidelines and policies of the University of Toronto's Animal Use Protocol.

**Peptide injection in rat brain.** Male Wistar rats (~300-350 g) were anaesthetized (ketamine 66 mg/kg, acepromazine 3 mg/kg and pentobarbital 22 mg/kg i.p.) for chronic stereotaxic implantation. A unilateral stainless steel guide cannula (G22) was placed into the centre of the

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left caudate putamen (ant. +1.5, lat. -2.2, vert. -5.0) (Paxinos and Watson, 1982) to allow peptide or vehicle injection. Alternatively, bilateral stainless steel guide cannulae (G22) were stereotaxically placed into the centre of left and right caudate putamen for simultaneous drug and vehicle injections. The guide cannula was kept patent by a stylet (Plastic Products Company, Roanoke, VA) terminating 0.5 mm below the guide tip, located 2 mm above the point of injection. The rats were allowed one week of postoperative recovery before experimental use. The stylets were withdrawn and injections (peptide or vehicle) made into conscious rats using a 28 gauge internal cannula connected by PE-50 polyethylene tubing to a gas-tight Hamilton syringe. For unilateral operated animals, a total of 2-3 µl volume of peptide in vehicle or vehicle alone was injected into the left striatum, followed 15 minutes later by a subcutaneous injection of 0.25 mg/kg apomorphine. Rats with bilateral cannulae were administered peptide into the left striatum and vehicle into the right striatum simultaneously, followed 15 minutes later by a subcutaneous injection of 0.25 mg/kg apomorphine.

**Ipsilateral rotation characterization.** The intensity of the behavioral response was assessed every 2-5 minutes post-injection. Asymmetry (ipsilateral to the side of peptide or vehicle injections) was scored on the 0-3 system. Animals showing an ability to move in right and left directions were not categorized as circling. However, an ability to circle in one direction (asymmetric body posture) was scored on a 0-3 response according to observations in the open field and to the lifting of the tail. The criteria which met the 0-3 scores were: 0=no asymmetry, response of animals the same as untreated rats; 1 = a distinct tendency for animals to move in one direction when handled but still capable of movement in either direction; 2 = spontaneous movements in one direction, a twisting of the body in this direction, exaggerated when handled,

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with inability to move in opposite direction; 3 = a marked and intense twisting of the body in one direction, active circling movements when disturbed, the animal being unable to move in the opposite direction.

**Autoradiography of ligand binding in rat brain.** Male Wistar rats implanted stereotactically with a cannula in the cerebroventricle were injected with D2-TM7 peptide (10 µg) or vehicle and were sacrificed 30 minutes later. The brains were rapidly resected, frozen on dry ice, and coronal sections (10 µm) through the caudate putamen (1.4 mm rostral to bregma) and nucleus accumbens (2.2 mm rostral to bregma) were obtained using a cryostat and thaw-mounted onto gelatin-coated slides. For the assay, the sections were air dried for 30 minutes and then preincubated with buffer (50 mM Tris-HCl (pH 7.4), 5 mM EDTA, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, and 120 mM NaCl), then incubated with [$^3$H]-nemonapride (0.492 nM) to label D2 dopamine receptors or [$^3$H]-SCH 23390 (1 nM) to label D1 dopamine receptors. Non-specific binding was defined by incubation with 1 µM (+)butaclamol. Rimcazole 1 µM, a selective σ receptor antagonist was added to the sections incubated with [$^3$H]-nemonapride to prevent its binding to σ receptors. After incubation for 90 min, the slides were washed in ice-cold buffer twice, dipped into ice-cold deionized water to eliminate salts, dried and exposed to Hyperfilm (Amersham) for 21 days. The autoradiographs were subjected to densitometric analysis (MCID, Imaging Research Inc.) and using radioisotopic standards, receptor densities were determined.

**Cardiovascular effects of TM peptide.** Male Wistar rats (250-270 g) were deprived of food for 12 hours prior to surgery, but received water ad libitum. For vascular access, rats were premedicated with atropine, anaesthetized with halothane and the right carotid artery and the left



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jugular vein were cannulated with PE 50 polyethylene catheters. The arterial line was used for blood pressure recording and the jugular vein was used for injection of drugs. The animals were allowed to recover for a minimum of 3 hours, so that the experiments were conducted in awake and non-restrained animals. The baseline mean arterial pressure and heart rate values were based on 30 consecutive minutes of stable readings. All drugs and vehicle were administered in a volume of 200 µl.

**Urine output following injection of a V2 vasopressin receptor TM peptide.** Male Wistar rats (250-270 g) were anaesthetized with halothane and the aorta and baldder were cannulated with PE 50 polyethylene catheters. The rats were unconscious and received a constant saline infusion before and during urine collection. Measurement of urine output volume started after urine flow stabilized. Urine was collected for 15 minutes prior to injection of the peptide and continued every 15 minutes until the conclusion of the experiment. A peptide derived from TM 7 of the V2 vasopressin receptor was injected 75 minutes after the injection of the vehicle.

**Microdialysis measurement of dopamine.** Rats were implanted with intracerebral guide cannulae (Plastic Products Company, Roanoke, VA, U.S.A.) for the microdialysis probes (Carnegie Medicin, Sweden) under anaesthesia as above. The cannula was inserted into the medial nucleus accumbens (shell region) (coordinates relative to bregma: A 1.5, L 1.3, V 7.6) or into the centre of the left caudate putamen (A +1.5, L. -2.2, V -5.0) and rats were allowed to recover for at least 5 days. On the day of the experiment, the guide cannula was replaced by the dialysis probe (2 mm, CMA/12, Carnegie Medicin, Stockholm, Sweden) and perfused with artificial cerebral spinal fluid (NaCl 145 mM, KCl 2.7 mM, $CaCl_2.2H_2O$ 1.2 mM, $MgCl_2$ 1.0

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mM, $Na_2HPO_4$ 2 mM, ascorbic acid 0.2 mM, pH 7.4) at a rate of 1 µl/min. Perfusion in the awake, unrestrained and mobile animals was continued for 3-4 hours until the basal efflux of dopamine and its metabolites (HVA, DOPAC) were stable for 3 consecutive measurements. The animals were then injected with cocaine 5 mg/kg i.p.. Twelve minutes after i.c.v. injection with 100 µg or 300 µg of DAT TM 12 peptide (n=3 at each dose tested) or vehicle 5 µl (n=8). Dialysate was collected over 30 minutes periods and injected directly into a HPLC system equipped with a Biophase ODS 5 µm, 4.6 x 250 mm column, Waters 590 pump with U6K injector and ESA 5100A electrochemical detector with Model 5011 analytical cell for measurement of DA, DOPAC and HVA. The mobile phase consisted of 50 mM sodium phosphate monobasic, 0.5 mM EDTA, 1.8 mM sodium octyl sulfonate, 14 % methanol, with pH adjusted to 3.50 with phosphoric acid. Sensitivity of dopamine detection was 2 pg. The percentage recovery of dopamine through the dialysis cannula was calculated each time.

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**Results**

The TM domains of GPCRs are predicted to exist as α-helices within the membrane (Liu and Deber, 1998), which in turn promotes packing and assembly to align the TM domains in a highly organized structure (Palczewski et al., 2000). To determine the structure of the present synthetic TM-based peptides, their circular dichroism (CD) spectra were recorded in the membrane-mimetic environment of sodium dodecylsulfate micelles, as shown in Figure 1. CD spectra of both the D2-TM7 and the β2-TM6 peptides at 25°C and 37°C displayed significant α-helical character, exhibiting minima near 208 and 222 nm. The fact that both peptides were α-helical in micelles under physiological salt and temperature conditions indicates their conformational potential to interact with other native α-helices. Further, structural correspondence has been observed for non-polar peptide segments of intact proteins, both in their ability to retain α-helical structure (Voss et al., 1993; Myers et al., 1997b; Wang and Deber, 2000), and to stabilize sequence-dependent helix-helix interactions within membranes (Myers et al., 1997a). It is highly conceivable therefore, that the synthetic TM peptides acted *in vivo* by mimicking the native TM domain morphology.

In an attempt to disrupt the intramolecular interactions among TM domains, a synthetic peptide derived from TM domain 7 of the D2 dopamine receptor was synthesized. Since the TM domains of a GPCR are arranged in a circular formation, with the TM domains 1 and 7 adjacent to each other (Suryanarayana et al., 1992; Strader et al., 1994), we hypothesized that a peptide based on one of these terminal TM domains would be the most effective at interacting with the receptor. The D2-TM7 peptide inhibited binding of the dopamine antagonist [$^{3}$H]-spiperone to membranes from cells expressing D2 receptors in a dose-dependent manner (Figure 2A). A peptide derived from the TM 5 of the D2 receptor also decreased binding, but to a lesser extent.

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Peptides derived from the TM 7 domain of the vasopressin V2 receptor, the TM 12 region of the dopamine transporter and TM 1 region of the $GABA_A$ receptor α subunit had negligible effect on [$^3$H]-spiperone binding to the D2 dopamine receptor. The D2-TM7 peptide also inhibited binding of the agonist [$^3$H]-quinpirole to the D2 dopamine receptor (Figure 2B) in a manner similar to a classical dopamine antagonist (Figure 2C), and prevented binding and incorporation of the D2-selective photoaffinity ligand [$^{125}$I]azidonemonapride to the D2 receptor species (Figure 2D). These results are consistent with a previous *in vitro* study that showed that a peptide derived from a TM domain of the D1 dopamine receptor acted as an irreversible inhibitor of D1 receptor function (George et al., 1998). Therefore, *in vitro*, treatment of D2 dopamine receptor with the D2-TM7 peptide resulted in the disruption of the ligand binding.

Receptor autoradiography of rat brain following intracerebroventricular (i.c.v.) injection of the D2-TM7 peptide revealed reduced ligand binding to the D2 dopamine receptor (Figure 3A, B). Blockade of D2 dopamine receptor binding was analogous to that achieved by the classical antagonist (+)-butaclamol (Figure 3C). Notably, when ligand binding to the D1 dopamine receptor was examined in animals treated with D2-TM7 peptide, there was no effect (Figure 3D-F), indicating that the peptide had a specific and selective antagonist effect *in vivo* on the ligand binding capability of the D2 receptor.

The function of the D2-TM7 peptide was tested *in vivo* for D2 receptor antagonist activity using an animal model for striatal function, established for screening dopamine receptor antagonists (Costall et al., 1983). Awake, freely mobile rats bearing stereotactically guided cannulae were injected into the left caudate nucleus with peptides derived from TM 7 of the D2 receptor or TM 6 of the $\beta_2$-adrenergic receptor. The injection of either peptide did not have an effect on the locomotion of the animal. However, following subcutaneous challenge with the

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dopamine analogue apomorphine 15 minutes later, an ipsilateral asymmetric body rotation in rats administered D2-TM7 peptide was observed, achieving a behavioral response score that was not observed in rats injected with the $\beta_2$-adrenergic receptor TM 6 peptide (Figure 4A). Similar ipsilateral asymmetric rotation was also documented in rats following bilateral intracaudate administration of D2-TM7 peptide and vehicle simultaneously into left and right caudate nucleus respectively (Figure 4B). These results indicate that the D2-TM7 peptide acted in the animal model as a functional D2 receptor antagonist.

To test the universal applicability of our hypothesis, the actions of peptides designed based on the sequences of other GPCRs were tested for effects to inhibit the activity of selected receptors. A peptide based on the TM 7 of the $\alpha_{1A}$-adrenergic receptor, if an effective $\alpha$-adrenergic antagonist, would be hypothesized to lower arterial blood pressure. Administration of an $\alpha_{1A}$ adrenergic receptor TM 7 peptide i.v. into rats implanted with carotid cannulae resulted in a reduction of systolic and diastolic blood pressure, with a concomitant rise in the heart rate (Figure 5A). In comparison, the $\alpha_{1A}$-adrenergic receptor antagonist prazosin also lowered blood pressure and elevated heart rate (Figure 5B). The effect of the $\alpha$-adrenergic agonist phenylephrine to elevate blood pressure was attenuated when administered after the $\alpha_{1A}$-adrenergic receptor TM 7 peptide (Figure 5C, D). In addition, a peptide based on the TM 7 of the $\beta_1$-adrenergic receptor was predicted to lower heart rate and was tested for effects on spontaneous cardiac activity and blood pressure in rats. With this peptide, a short-lived but marked slowing of heart rate was revealed with a selective drop in diastolic blood pressure (Figure 5F).

It was then hypothesized that a peptide derived from TM 7 of the V2 vasopressin receptor could block the actions of vasopressin (antidiuretic hormone) on the V2 vasopressin receptor and

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therefore act as a diuretic. Injection of peptide vehicle through an aortic cannula in a rat resulted in a slight increase in urine output compared to measurements made prior to injection (Figure 6). However, the *in vivo* administration of V2-TM7 peptide resulted in diuresis much greater than that seen with vehicle injection. These observations suggested that the V2 vasopressin receptor TM 7 peptide was disrupting the antidiuretic function of the V2 vasopressin receptor.

In order to determine whether the principle of TM domain interference with receptor function could be extrapolated to other membrane proteins with multiple membrane-spanning domains, a peptide based on one of the terminal TM domains of the DAT, TM 12, was synthesized. The peptide was used in an experiment wherein cocaine-induced dopamine release from nucleus accumbens and caudate nucleus of live rats could be reliably measured by a stereotactically implanted microdialysis probe. It was demonstrated that pretreatment with the 100 μg or 300 μg DAT TM 12 peptide resulted in complete suppression of the cocaine-induced dopamine release detected in vehicle treated control animals (Figure 7), indicating functional antagonism of cocaine action. It was not clear if this blockade was due to purely to an inhibition of cocaine binding, purely a disruption of transporter activity, or a combination of both actions. Interestingly, administration of 100 μg of the DAT TM 12 peptide alone (Figure 7A) had no effect on basal dopamine release from caudate nucleus or nucleus accumbens. However, administration of 300 μg of the DAT TM 12 peptide alone produced a gradual increase in basal dopamine release (Figure 7B) as well as a similar increase in basal DOPAC and HVA level (data not shown). Therefore, it appeared that the peptide interfered with transporter activity and did not solely inhibit cocaine binding.

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## Discussion

In this report, we postulated that peptides derived from the TM domains of GPCRs and monoamine transporters, when administered *in vivo*, may disrupt the function of the protein from which they are derived. To test this hypothesis, we employed well-characterized behavioural and physiological endpoints and tested several GPCR-derived TM peptides and a TM peptide based on the DAT. We demonstrated that, when a peptide derived from a transmembrane domain of the D2 dopamine receptor was injected into caudate nucleus of conscious, freely-mobile rats and challenged with apomorphine, a rotational behavior resulted, indicating D2 receptor blockade. This effect was not seen in rats injected with a similar peptide derived from the $\beta_2$-adrenergic receptor. The i.v. administration of a TM peptide derived from the $\alpha_1$-adrenergic receptor resulted in lowered arterial blood pressure and injection of a $\beta_1$-adrenergic receptor peptide resulted in decreased heart rate, suggesting that these peptides behaved as anatgonists for the $\alpha_1$- and $\beta_1$-adrenergic receptors respectively. We then showed that *in vivo* antagonism of vasopressin activation of the V2 vasopressin receptor by a V2 receptor-derived TM peptide. Finally, we demonstrated that dopamine release from caudate nucleus and nucleus accumbens in rats following cocaine administration was blocked by the DAT-based TM peptide.

This study provides evidence for the first time *in vivo*, that peptides derived from the TM domains of GPCRs and monoamine transporters may, by a novel mechanism of functional disruption, represent an atypical type of antagonist for these proteins. We propose that the mechanism of action of the TM-based peptides involves their specific interactions with complementary TM domains/segments within the integral membrane protein, thereby competing for the normal intramolecular interactions of the native TM domains (Baldwin et al., 1997). As we have shown by circular dichroism spectroscopy, exogenous TM peptides retain $\alpha$-helical

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properties similar to that proposed for native transmembrane domains. Not surprisingly therefore, TM domain-derived peptides have been shown to be good predictors of helix-helix interactions in membrane proteins (Wang and Deber, 2000; Melnyk et al., 2001; Therien and Deber, 2002). Selective stabilization of helices by neighboring helices, with significant, but not unbreakable, anchoring forces between particular adjacent helices has been demonstrated for rhodopsin (Palczewski et al., 2000) and the structurally analogous membrane protein bacteriorhodopsin (Oesterhelt et al., 2000). These interactions contribute to the proper three-dimensional folding and conformation of the protein and in the creation of the ligand binding pocket, often deep within the TM core. We predict that integral membrane proteins having one or more TM domains will be susceptible to disruption of their structure and therefore function, by peptides having the amino acid sequence of a TM segment that participates in forming the ligand-binding crevice. An antagonist peptide having the amino acid sequence of the TM domain of a particular integral membrane protein would likely show sequence-dependent specificity for that protein and, as demonstrated in the present work, may not interfere with the function of related proteins. Admittedly, there are obvious problems from the clinical viewpoint in using peptides such the ones that were studied in this report as therapeutic agents. For example, drug delivery to the site of action and the longevity/stability present major challenges in developing effective peptide drugs (Bickel et al., 2001; Torchilin and Lukyanov, 2003). Nevertheless, the potential advantages in being highly selective to a particular class or subtype of receptor (or proteins with multiple membrane-spanning helices) obviously make the targeting of intramembrane interactions very intriguing.

In conclusion, we and others have previously shown disruption of GPCR function *in vitro* by peptides derived from TM domains of these receptors. Now, in this study, we have shown by

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CD spectra that TM peptides based on TM domains of GPCRs, when solubilized in a membrane-mimetic environment, have helical structures like native TM domains. We demonstrated that these TM peptides can elicit behavioural and physiological effects *in vivo* by selectively inhibiting the function of the receptor from which it was derived. Further, we have expanded the concept and shown that other multiple TM domain proteins such as the DAT can also be disrupted *in vivo* by a peptide based on one of the TM helices of the protein. Our findings may provide a key to developing a novel category of therapeutic agents that disrupts the hydrophobic core architecture of integral membrane proteins and may be of benefit in the rational design of drugs targeting these proteins.

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**Footnotes**

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## Figure Legends

**Figure 1.** CD spectra of the synthetic D2-TM7 and β2-TM6 peptides at varying temperatures. 40 μM of each peptide, solubilized as described in the Methods section, was diluted in buffer containing 50 mM SDS, 10 mM Tris-HCl (pH 7.4), 2 mM EDTA, with NaCl 100 mM. The result shown is representative of the three replicates of this experiment that were performed.

**Figure 2.** Effect of TM peptides on radioligand and photoaffinity ligand interaction with the D2 dopamine receptor. (A) Inhibition by transmembrane domain peptides of $[^3H]$-spiperone binding to the dopamine D2 receptor expressed in Sf9 cells. The peptides correspond to the sequences respectively of the dopamine D2 receptor TM 7 (D2 TM7) and TM 5 (D2 TM5); the V2 vasopressin receptor TM 7 (V2 TM7), the $GABA_A$ receptor subunit α TM 1 (GABA TM), and the dopamine transporter TM 12 (DAT TM12). The effect of the peptides is shown as the mean ± SEM percentage of the total $[^3H]$spiperone binding from three independent experiments. Statistically significant differences, as determined by t-test, are noted by asterisks. Dose-dependent competition for $[^3H]$-quinpirole binding to the D2 dopamine receptor expressed in Sf9 cells by (B) D2-TM7 peptide and (C) spiperone. The data shown is representative of three replicates. (D) Effect of D2-TM7 peptide on $[^{125}I]$-azidonemonapride labeling of D2 receptors expressed in Sf9 cells. Photoaffinity labeling of D2 receptors monomers (~48 kDa), dimers (~98 kDa) and tetramers (~200 kDa) was visualized (Lane 1) that was prevented by prior incubation with the D2 TM7 peptide (1 μg/ml) (Lane 2) or (+)-butaclamol (1 μM) (Lane 3). The autoradiogram shown is representative of the five identical photoaffinity labelling experiments performed.

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**Figure 3.** Autoradiography of D1 and D2 dopamine receptors in coronal sections of rat brain following administration of D2-TM7 peptide or vehicle i.c.v.. Rat brain sections showing binding of [$^3$H]-nemonapride (0.492 nM) to D2 dopamine receptors (A, B, C) or [$^3$H]-SCH 23390 (1 nM) to D1 dopamine receptors (D, E, F). Sections are from rats treated with vehicle (A and D) or the D2-TM7 peptide (B and E). Non-specific binding was defined by incubation of the vehicle-treated sections with 1 μM (+)butaclamol (C and F). Abbr.: CP = caudate putamen, Acb = nucleus accumbens, OT = olfactory tubercle. The data shown is representative of six replicates.

**Figure 4.** Rotational activity induced by administration of TM peptides into the caudate nucleus of rats. (A) Apomorphine-induced asymmetric body rotation following unilateral (left side) administration of D2-TM7 peptide or β2-TM6 peptide into caudate nucleus. The duration and extent (score) of the asymmetric body response after injection of D2-TM7 peptide (15 ng/3 μl) and β2-TM6 peptide, (15 ng/3 μl) are shown. (B) Apomorphine-induced asymmetric body rotation following bilateral simultaneous administration into caudate nucleus of D2-TM7 peptide (left side) and vehicle (right side). The duration and extent of asymmetric body response of a rat with bilateral cannulae after injection of D2-TM7 peptide (15 ng/3 μl) and vehicle (3 μl) concurrently are shown. The data shown is representative of three replicates.

**Figure 5.** Arterial pressure and heart rate recording from carotid artery cannulated rats following administration of TM peptides based on adrenergic receptors. Panels show effects of (A) the α1-adrenergic receptor TM 7 peptide and (B) prazosin. Effects of (C) phenylephrine, (D) phenylephrine after $\alpha 1_A$–adrenergic receptor TM 7 peptide, (E) vehicle and (F) β1-adrenergic

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receptor TM 7 peptide are shown. Peptide or drug was administered at time 0. The data shown is representative of three replicates.

**Figure 6.** Urine output for bladder-cannulated rat following aortic injection of peptide vehicle and a peptide derived from TM domain 7 of the V2 vasopressin receptor. Injection of the peptide vehicle was performed 15 minutes after the initial urine output measurement and the time of the injection was considered time 0. The peptide was administered 75 minutes after the vehicle. Injection times are shown by arrows. The data shown is representative of three replicates.

**Figure 7.** In vivo microdialysis measurement of dopamine release after cocaine administration. Release of dopamine from shell of nucleus accumbens was monitored following cocaine administration with and without pretreatment with (A) 100 μg or (B) 300 μg of DAT TM 12 peptide. Cocaine (5 mg/kg) was injected i.p. at time 0 and vehicle or DAT TM 12 peptide was injected i.c.v. at – 12 min. The data shown is representative of three replicates.

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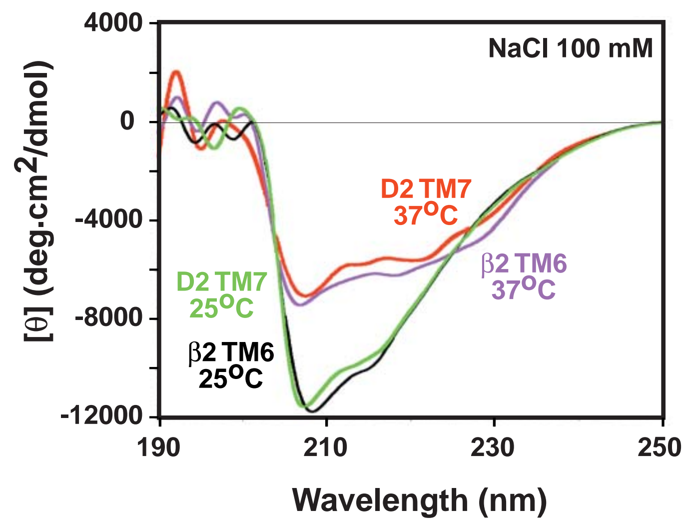
# Figure 1

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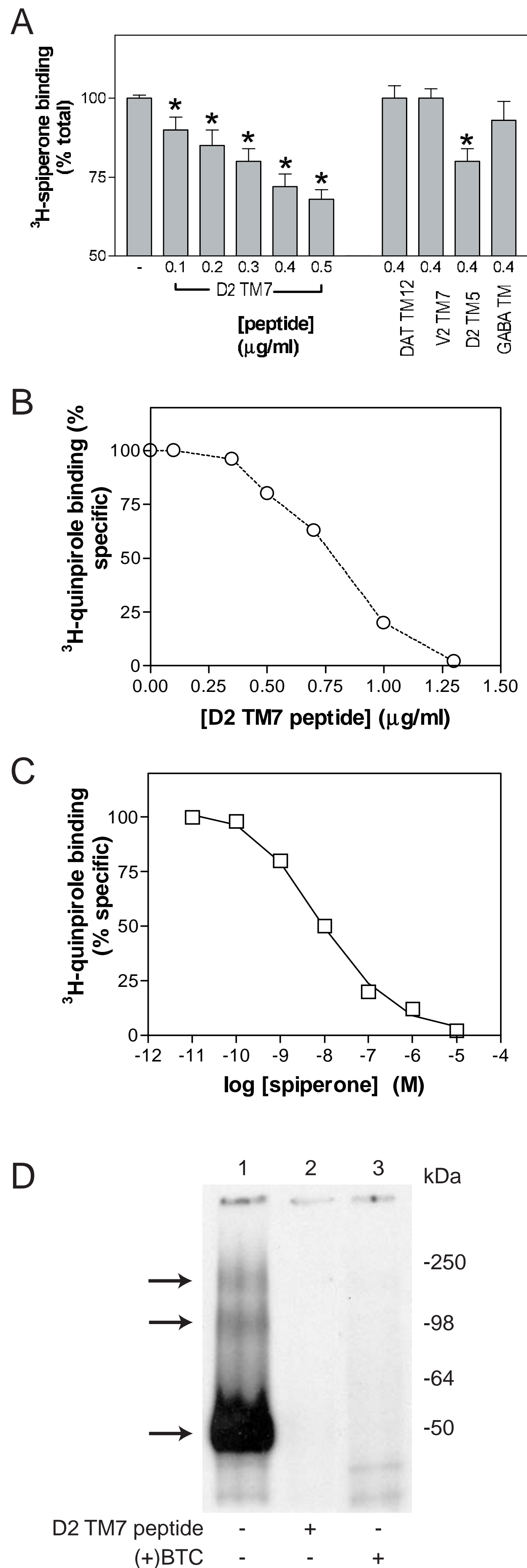
# Figure 2

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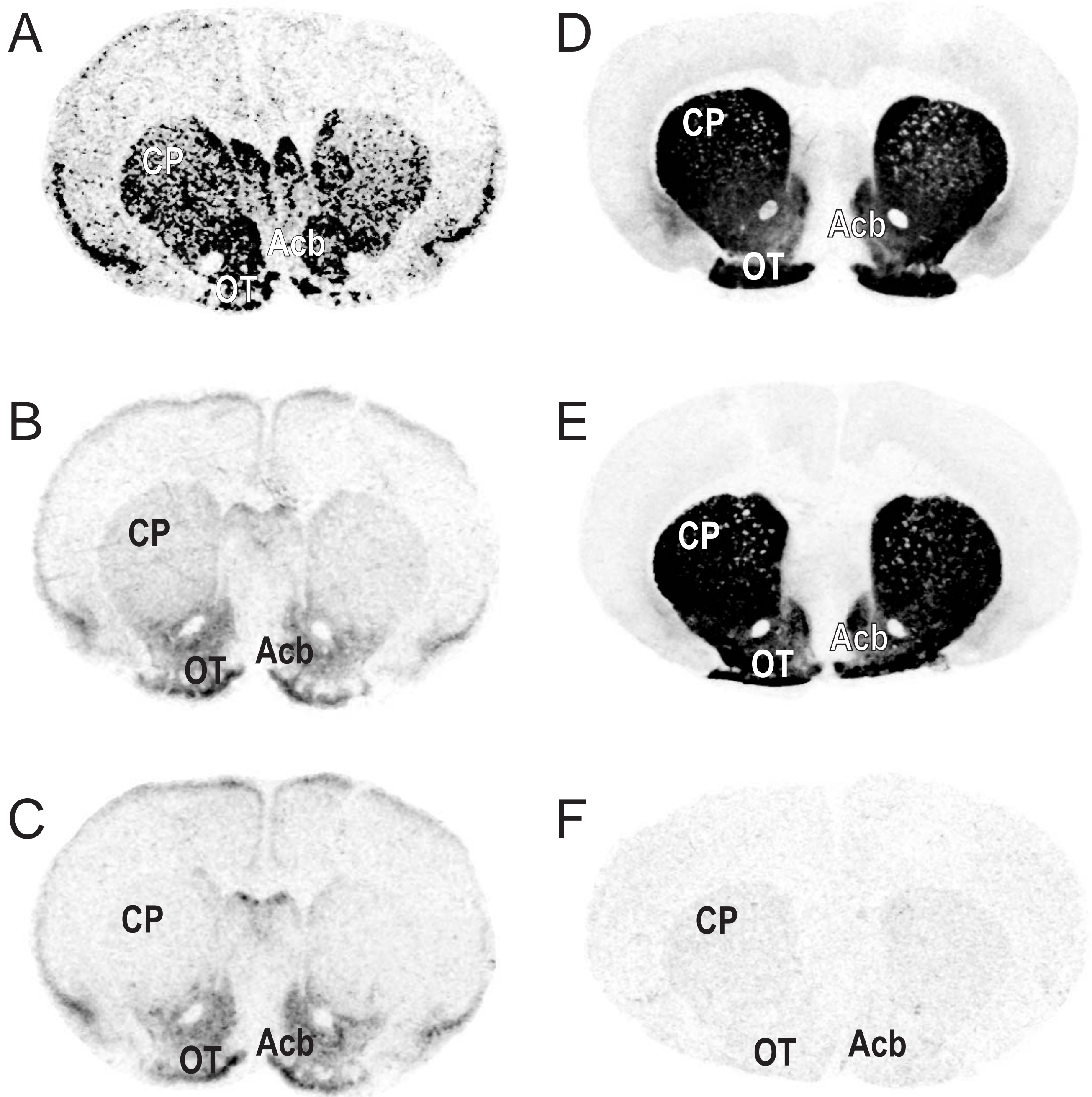
# Figure 3

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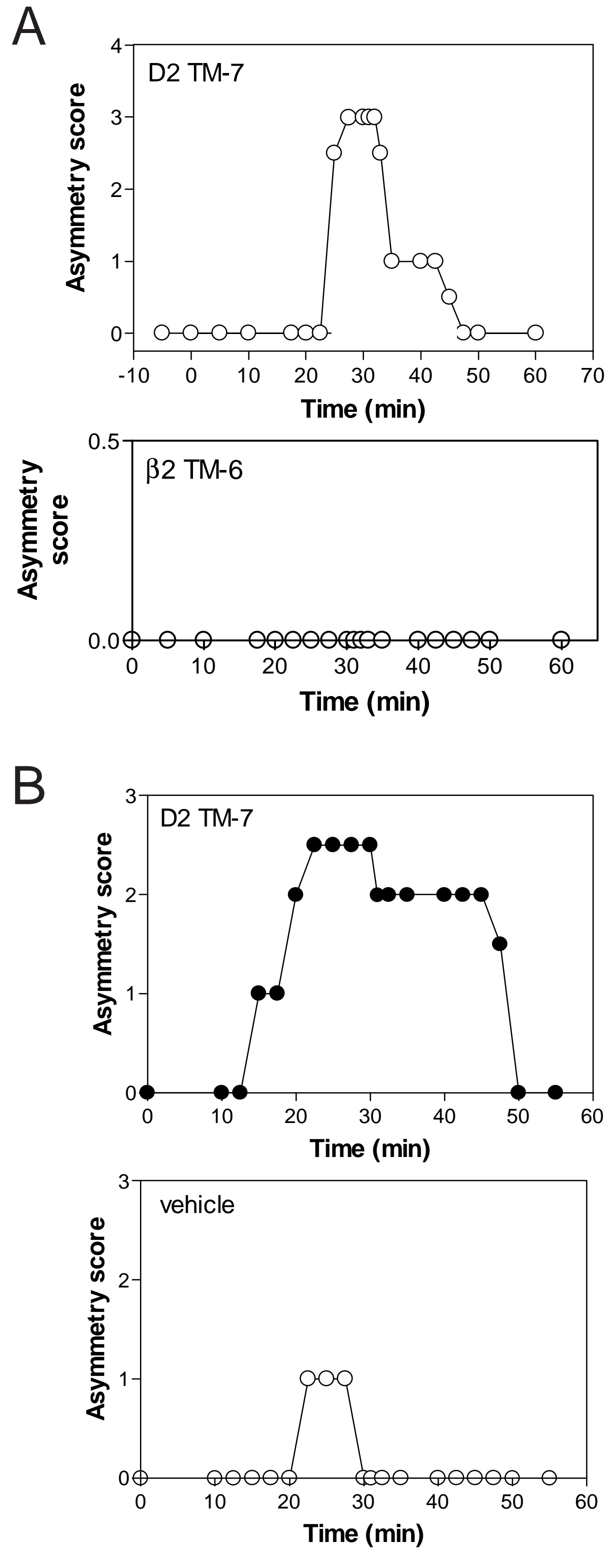
# Figure 4

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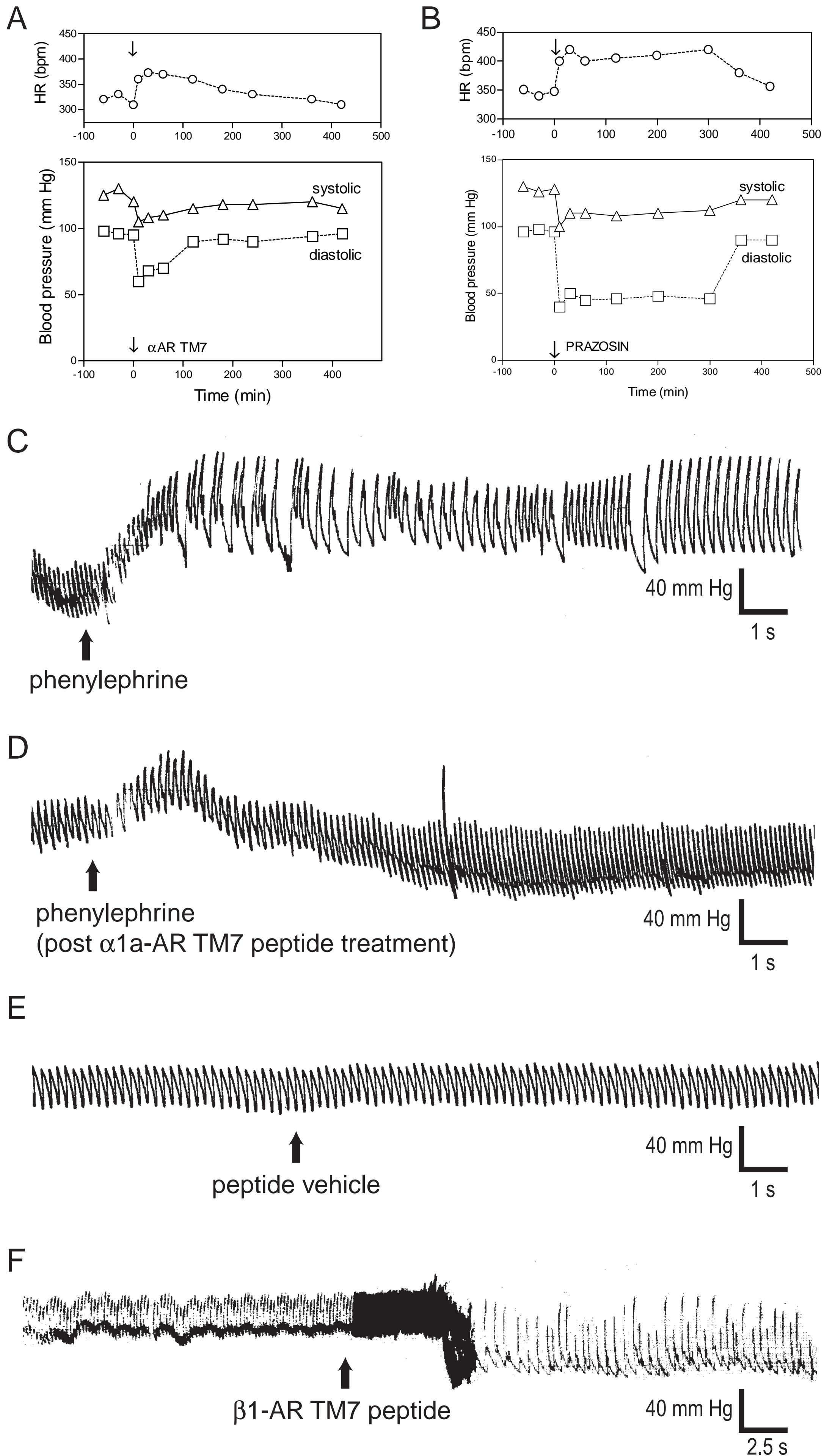


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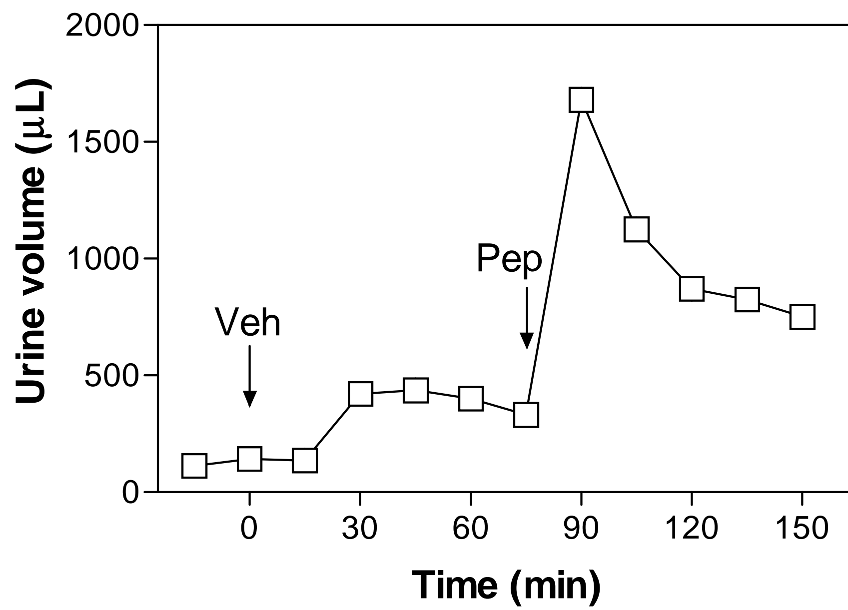
# Figure 6

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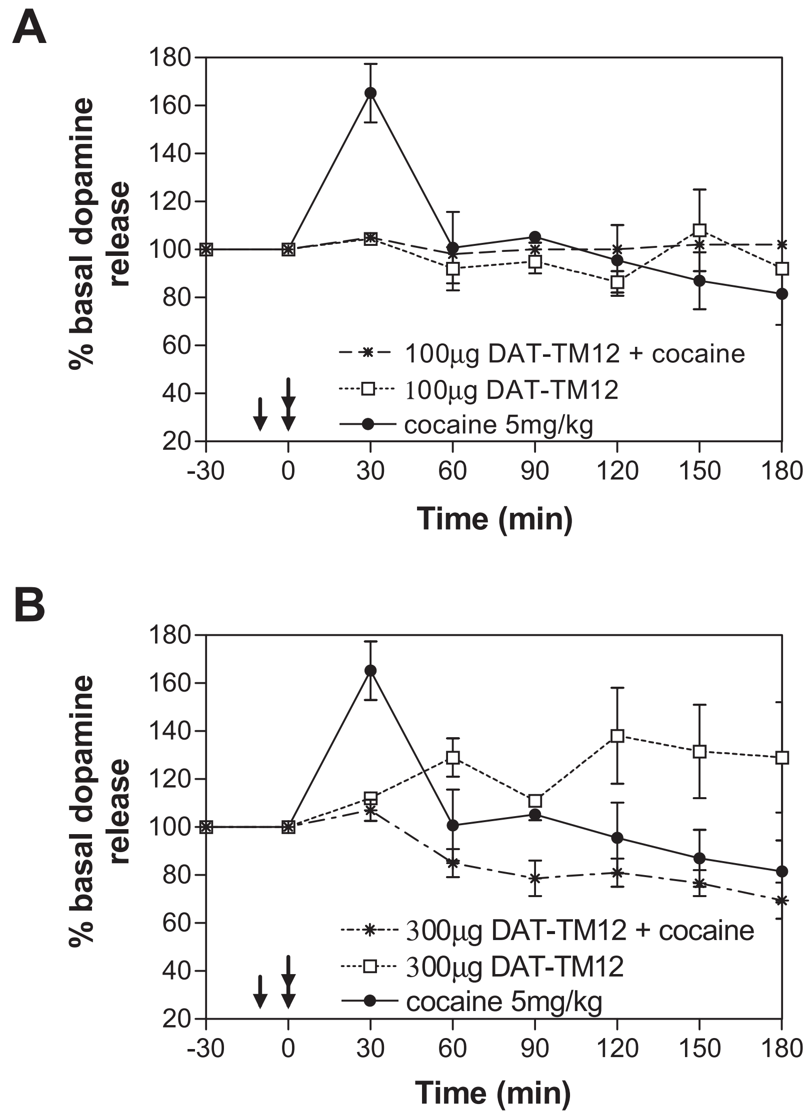


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