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Modulation of cellular activity and synaptic transmission in the ventral tegmental area

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Abstract

The mesolimbic dopamine system, of which the cell bodies are located in the ventral tegmental area, has been implicated in the physiology of reward and the related pathophysiology of drug abuse. This area has been a site of significant interest to study the effects of drugs of abuse and neurotransmitter systems implicated in the rewarding effects of these compounds. One important aspect of synaptic transmission is the ability of synapses to strengthen or weaken their connection as a consequence of synaptic activity. Recently, it has become apparent that this phenomenon is also present in the ventral tegmental area and that this may bear important functional consequences for the ways in which drugs of abuse assert their effect. Here, we will review the effects of neurotransmitter systems and drugs of abuse on cellular activity and synaptic transmission in the ventral tegmental area.

Keywords: Ventral tegmental area; Mesolimbic; Synaptic transmission; Electrophysiology; Drug addiction

1. Introduction

1.1. The mesencephalic dopamine system

The mesencephalic dopamine system has been divided into two main functional units, the nigrostriatal and the mesocorticolimbic system. The nigrostriatal pathway originates in the substantia nigra (A9) and projects to the dorsal striatum. It is involved in motor functioning and in the pathophysiology of Parkinson's disease and Huntington's chorea. The mesocorticolimbic pathway originates in the ventral tegmental area (A10) and projects to several limbic and cortical structures, including the nucleus accumbens, and prefrontal cortex. This pathway is implicated in the physiology of reward and motivation, the pathophysiology of drug abuse (Wise, 1998) and schizophrenia. This review focuses on the ventral tegmental area and its projections to the nucleus accumbens.

Dopaminergic neurons in the ventral tegmental area can switch from rhythmic firing to burst firing when the animal is presented with certain salient stimuli, for example food or water (Miller et al., 1981; Overton and Clark, 1997). This switch to burst firing results in increased dopamine release in target areas, leading to arousal and a behavioural response of the animal (Gonon, 1988; Suaud-Chagny et al., 1992). The recent finding that dopamine levels in the nucleus accumbens increase both before and after lever pressing for cocaine (Phillips et al., 2003) indicates that dopamine systems are involved in the anticipatory response prior to reward as well as mediating the reinforcing response after receiving the reward. An increase of dopamine levels in the nucleus accumbens is a common feature shared by many drugs of abuse (Di Chiara and Imperato, 1988). One striking difference between in vitro and in vivo recordings is that principal cells do not exhibit spontaneous burst firing in vitro (Johnson and North, 1992b). This shows the importance of input to these neurons in the control of firing, which is lost as a result of slice preparation. Activation of *N*-methyl-D-aspartate NMDA receptors, metabotropic glutamate receptors or blockade of specific K^+ conductances elicits burst firing in vitro (Johnson et al., 1992b; Johnson and Seutin, 1997; Seutin et al., 1993; Zheng and Johnson, 2002).

We will review the effects of different 'classical' neurotransmitters (dopamine, 5-hydroxytryptamine (5-HT), acetylcholine, noradrenaline, glycine and opioids), which affect

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neurons and synaptic transmission in the ventral tegmental area. Over the last few years, the existing knowledge on how classical neurotransmitter systems affect cells in the ventral tegmental area has been extended with information of additional neurotransmitters (adenosine-5-triphosphate (ATP), adenosine, histamine, nociceptin/orphanin FQ, orexins and endocannabinoids) having an effect in the ventral tegmental area.

1.2. Cell types in the ventral tegmental area

In the early 1970s, the first in vivo extracellular single-unit recordings were done in the ventral tegmental area (Bunney et al., 1973). Dopaminergic neurons in the ventral tegmental area, like in the substantia nigra, could be distinguished from non-dopaminergic cells based on their electrophysiological characteristics, like firing frequency and pattern, action potential characteristics and responsiveness to dopamine (Bunney et al., 1973). The first in vitro sharp-electrode intracellular recordings were done almost two decades later and many of the in vivo characteristics of dopaminergic cells could also be seen in vitro (Johnson and North, 1992b). Dopaminergic neurons, termed principal neurons, hyperpolarise in response to dopamine but not to met-enkephalin. Non-dopaminergic cells, termed secondary cells, do not respond to dopamine but hyperpolarise in response to met-enkephalin (Johnson and North, 1992b). The secondary neurons are thought to be mainly γ-amino butyric acid (GABA)ergic interneurons and GABAergic projection neurons (Carr and Sesack, 2000a; Nagai et al., 1983; Steffensen et al., 1998; Van Bockstaele and Pickel, 1995). The action potentials of principal cells are relatively slow compared to secondary cells and are followed by a much larger undershoot, which slowly depolarises back to the action potential threshold causing rhythmic firing of the cell (Bunney et al., 1973; Johnson and North, 1992b). This depolarisation is at least partly caused by a hyperpolarisation-activated depolarising current, I_h, which is present in principal cells but not in secondary cells (Johnson and North, 1992b). There is debate about the legitimacy of using the presence of an I_h as criterion for establishing whether one is recording from a principal cell. In some but not all studies, immunocytochemical analysis was performed to identify the nature of the recorded cell. There is not a complete overlap between cells possessing an I_h and labelling for tyrosine hydroxylase, an important enzyme in the dopamine synthesis pathway (Cameron et al., 1997; Jones and Kauer, 1999). This therefore means that there are dopaminergic neurons that do not have an I_h. A subset of neurons, termed tertiary neurons, hyperpolarises in response to dopamine and met-enkephalin as well as to 5-HT (Cameron et al., 1997). An I_h was found in 13% of these neurons (Cameron et al., 1997) and the identity of these neurons is as yet uncertain. Despite these considerations, the presence or absence of a prominent I_h is most commonly used to distinguish between principal and secondary neurons in in vitro preparations.

1.3. Modulation of synaptic transmission

Modulatory neurotransmitters and drugs of abuse can affect the mesolimbic system at the level of the ventral tegmental area and the nucleus accumbens. In this review, we will mainly look at direct in vitro effects of neurotransmitters within the ventral tegmental area, indicating the important indirect effects through other brain areas where relevant. Within the ventral tegmental area, neurotransmitters can have their effect directly on principal neurons, either by opening or closing specific ion channels or through second messenger systems (Fig. 1A). Alternatively, neurotransmitters or drugs of abuse might act specifically on secondary neurons and thereby modulate the inhibitory input onto principal neurons (Fig. 1B). Certain compounds act both at principal and secondary neurons. The overall effect in this case will depend on which action is predominant. Another way of influencing the excitability of neurons is by altering the amount of excitatory and inhibitory input onto the neuron (Fig. 1C). The level of excitation and inhibition can be changed by two forms of activity-dependent synaptic plasticity, long-term potentiation and long-term depression. In addition, other neurotransmitters can modulate the effect of excitation and inhibition by affecting the release of glutamate and GABA from the presynaptic terminal. This is another important site where neurotransmitters can regulate the state of neurons within the ventral

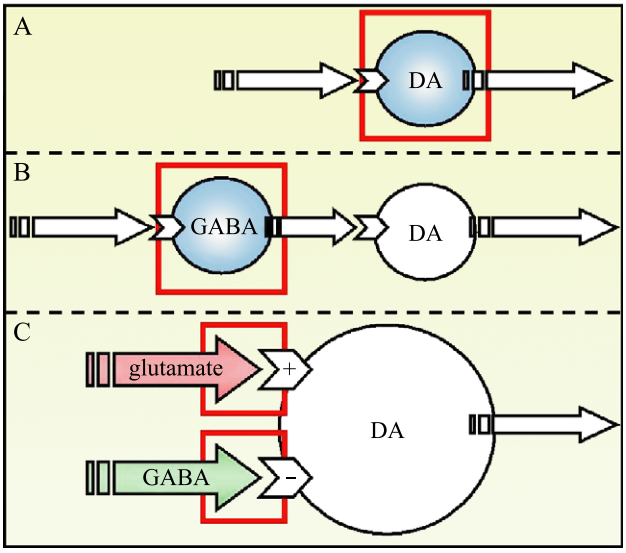

Fig. 1. Sites of modulation by neurotransmitters. Neurotransmitters can have their effect in the ventral tegmental area at a number of different sites. (A) On principal, dopaminergic, neurons by opening or closing specific ion channels or through second messenger systems. (B) On secondary neurons and thereby modulate the inhibitory input onto principal neurons. (C) By regulating the amount of excitatory and inhibitory input onto the neurons in the VTA.

tegmental area and where drugs of abuse can assert their effect.

We will first review the excitatory and inhibitory input to the principal neurons in the ventral tegmental area. Subsequently, synaptic plasticity in the ventral tegmental area and the role that drugs of abuse have in this phenomenon will be discussed. Finally, we will discuss the effects of other neurotransmitters on (i) principal neurons, (ii) secondary neurons and (iii) synaptic inputs.

The ventral tegmental area receives numerous inputs that can modulate the eventual output of the ventral tegmental area. First of all, excitatory and inhibitory afferents synapse on neurons and these inputs essentially drive the activity of ventral tegmental area neurons. These synaptic inputs display activity-dependent plasticity that is of relevance for the action of drugs of abuse. In addition, the activity of the ventral tegmental area is controlled by modulatory neurotransmitters (catecholamines, amino acids, neuropeptides and endocannabinoids). The ventral tegmental area contains multiple cell types that can be divided into principal dopaminergic and secondary GABAergic neurons. Modulatory input can have its effect at the cellular and/or synaptic level. These isolated effects can best be studied in the in vitro slice preparation. The aim of the present review is to summarise the results from in vitro studies and discuss these results with respect to the overall effect modulatory neurotransmitters will have.

2. Excitatory and inhibitory input

2.1. Glutamate

The ventral tegmental area receives glutamatergic input from a number of different brain structures, including the prefrontal cortex (Carr and Sesack, 2000b; Sesack and Pickel, 1992; Thierry et al., 1983), the pedunculopontine nucleus (Charara et al., 1996; Kelland et al., 1993) and the bed nucleus of the stria terminalis (Georges and Aston-Jones, 2001, 2002). Glutamate enhances the firing of principal ventral tegmental area neurons in vivo (Suaud-Chagny et al., 1992) and is crucial for the bursting activity of principal neurons through activation of NMDA receptors (Chergui et al., 1993; Gariano and Groves, 1988; Johnson et al., 1992b; Zhang et al., 1992; for review, see Overton and Clark, 1997).

As a result of this increased firing and switch to burst firing, glutamate injection into the ventral tegmental area increases dopamine levels in the nucleus accumbens (Kalivas et al., 1989; Suaud-Chagny et al., 1992) and increases locomotor activity (Kalivas et al., 1989; Pycock and Dawbarn, 1980). Besides influencing the activity of principal neurons, glutamatergic input to the ventral tegmental area is important in the context of synaptic plasticity and behavioural sensitisation. This will be discussed later on.

Glutamate can act on ionotropic glutamate receptors, which can be divided into NMDA and non-NMDA receptors, and on metabotropic glutamate receptors which are G-protein coupled. Excitatory synaptic potentials are mediated by NMDA and non-NMDA receptors in principal neurons (Johnson and North, 1992b). Activation of both NMDA and non-NMDA ionotropic glutamate receptors increased the firing rate of principal neurons (Mercuri et al., 1992; Seutin et al., 1990; Wang and French, 1993). In these studies, no burst firing of principal neurons was observed upon application of NMDA. In another study, burst firing of principal neurons could however be induced by the application of NMDA (Johnson et al., 1992b). The induction of burst firing by NMDA is facilitated by blockade of a Ca^{2+} activated K^+ conductance (Johnson and Seutin, 1997; Seutin et al., 1993).

Secondary neurons depolarise in response to both NMDA and non-NMDA ionotropic glutamate receptor agonists (Wang and French, 1995) and also show NMDA and non-NMDA receptor mediated synaptic potentials (Johnson and North, 1992b).

Activation of group I (metabotropic $mglu_1$- and metabotropic $mglu_5$) receptors on principal neurons produces both a sodium-dependent excitatory response (Mercuri et al., 1993; Shen and Johnson, 1997) and a later described slow apamin-sensitive inhibitory response (Fiorillo and Williams, 1998). Activation of metabotropic receptors also facilitates burst firing (Zheng and Johnson, 2002). Secondary neurons also depolarise in response to metabotropic glutamate receptor agonists, through activation of group I metabotropic glutamate receptors (Zheng and Johnson, 2003a).

Recently, the role of presynaptic metabotropic glutamate receptors on excitatory transmission was examined. Both an agonist for groups I and II as well as an agonist for group III metabotropic glutamate receptors reduced glutamate release onto principal ventral tegmental area neurons (Zheng and Johnson, 2003b). This reduction in glutamate release mainly inhibited the NMDA-mediated post-synaptic response whilst affecting the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-mediated response to a lesser extent (Zheng and Johnson, 2003b). Metabotropic glutamate receptors present on GABAergic nerve terminals in the ventral tegmental area are thought to regulate the release of GABA onto principal neurons (Zheng and Johnson, 2003a).

Overall, the glutamatergic input is crucial in determining the firing pattern (and thereby the dopaminergic output) of the principal cells in the ventral tegmental area.

2.2. GABA

There is indication that the non-dopaminergic neurons are not all local interneurons but that a subset of GABAergic neurons originating in the ventral tegmental area projects to other brain areas, including the nucleus accumbens, providing a non-dopaminergic output pathway

(Steffensen et al., 1998; Van Bockstaele and Pickel, 1995).

Besides local inhibition, there are also GABAergic projections to the ventral tegmental area from other brain areas. The main GABAergic input to the ventral tegmental area originates in the nucleus accumbens though this is not the only GABAergic projection to the ventral tegmental area (Waddington and Cross, 1978). Another suggested origin of GABAergic innervation to the ventral tegmental area is the ventral pallidum (Groenewegen et al., 1993).

GABA causes a hyperpolarisation by acting either at $GABA_A$ or $GABA_B$ receptors. The hyperpolarisation caused by $GABA_A$ receptor activation is the result of the opening of a Cl^- channel whilst the $GABA_B$ response is the result of a K^+ conductance.

In the ventral tegmental area, both principal and secondary neurons receive GABAergic input (Johnson and North, 1992b) and both the principal and secondary neurons show $GABA_A$ receptor mediated responses, which is in contrast to other aminergic brain areas (Johnson and North, 1992b). The $GABA_A$-mediated synaptic responses in principal neurons occurred spontaneously and were tetrodotoxin-sensitive and therefore are the result of spontaneous action potential firing of GABAergic neurons in the slice (Johnson and North, 1992a). The fact that $GABA_B$ responses did not occur spontaneously in principal neurons, were unrelated to $GABA_A$-mediated events and were inhibited by different agonists acting at presynaptic muscarinic and 5-HT receptors led to the notion that there were distinct afferents mediating the $GABA_A$ and $GABA_B$ response (Sugita et al., 1992).

Activation of $GABA_B$ receptors on presynaptic glutamatergic nerve terminals suppresses excitatory transmission onto secondary neurons but not onto principal neurons (Bonci and Malenka, 1999, but see Wu et al., 1999). This provides a mechanism by which GABA can regulate excitatory input onto secondary neurons independently of excitatory input onto principal neurons.

Activation of GABAergic receptors in the ventral tegmental area would most likely lead to inhibition of the dopaminergic output. Recently, it has been shown that systemic or local administration of a $GABA_B$ receptor agonist reduced the firing rate and bursting activity of principal neurons indicating indeed an overall inhibitory effect of these compounds (Erhardt et al., 2002).

In summary, GABA can have not only a direct inhibitory effect on principal neurons but also an indirect excitatory effect by disinhibiting secondary neurons.

3. Synaptic plasticity in the ventral tegmental area

Since the early 1990s, a link has been suggested between behavioural sensitization, which is a model for the increased sensitivity for drugs of abuse on subsequent exposures (Robinson and Berridge, 1993), and glutamatergic neurotransmission (Karler et al., 1991; Vanderschuren and Kalivas, 2000). Excitatory connections from the prefrontal cortex to the ventral tegmental area are important for developing behavioural sensitization (see Wolf, 1998 for review). In these early studies, it was speculated that activity-dependent synaptic plasticity might play a crucial role in this process. It was not however until the late 1990s that the occurrence of synaptic plasticity in the ventral tegmental area was first reported (Bonci and Malenka, 1999). The most interesting finding of this study was that whilst at excitatory synapses onto principal neurons long-term potentiation could be elicited with a pairing protocol, this was not possible at excitatory synapses onto GABAergic neurons (Bonci and Malenka, 1999). Long-term potentiation onto principal neurons is NMDA-dependent and mglu receptor-independent (Bonci and Malenka, 1999; Overton et al., 1999). So long-term potentiation can be elicited at excitatory synapses in the ventral tegmental area.

What about long-term depression? Two studies not long after the discovery of long-term potentiation in the ventral tegmental area showed that long-term depression can also be elicited at excitatory synapses onto principal cells in the ventral tegmental area (Jones and Kauer, 1999; Thomas et al., 2000). This long-term depression is both NMDA- and metabotropic glutamate receptor-independent, as well as L-type Ca^{2+} channel-independent and can be induced by repetitive depolarisation of the principal neuron (Jones and Kauer, 1999; Thomas et al., 2000). A rise in intracellular Ca^{2+} is required to obtain this long-term depression (Jones and Kauer, 1999; Thomas et al., 2000). Dopamine prevented the induction of long-term depression and further pharmacological experiments showed that this was the result of D2-like receptor activation (Jones and Kauer, 1999; Thomas et al., 2000). Activation of D2-like receptors blocks N- and P/Q-type Ca^{2+} currents in principal neurons indicating an involvement of one or both of these Ca^{2+} channels (Cardozo and Bean, 1995; Jones and Kauer, 1999; Thomas et al., 2000).

So, glutamatergic transmission onto principal neurons shows both long-term potentiation and long-term depression, allowing a bi-directional control of synaptic strength. The next question was whether drugs of abuse could affect these forms of activity-dependent synaptic plasticity. If long-term potentiation and long-term depression are important in mediating the effects of drugs in abuse and in particular represent the physiological correlate of behavioural sensitization then drugs of abuse should affect long-term potentiation and long-term depression.

The first drug of abuse to be examined was amphetamine and its effect on the induction of long-term depression in the ventral tegmental area. Amphetamine blocked the induction of long-term depression, providing the first example of a drug of abuse influencing synaptic plasticity in this area (Jones and Kauer, 1999). Nicotine application increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) onto principal neurons (Mansvelder and McGe-

hee, 2000). This increase in spontaneous excitatory postsynaptic currents enabled a depolarisation of the postsynaptic neuron to induce long-term potentiation. In the absence of nicotine, presynaptic stimulation was required alongside postsynaptic depolarisation to induce long-term potentiation (Mansvelder and McGehee, 2000). The nicotine-induced increase in spontaneous excitatory postsynaptic current frequency shows a clear correlation with the amount of long-term potentiation induced (Mansvelder and McGehee, 2000). Activation of the postsynaptic nicotinic receptor was not sufficient to induce long-term potentiation, though it did increase the firing frequency of the principal neurons (Mansvelder and McGehee, 2000). Despite not being sufficient to induce long-term potentiation, this postsynaptic effect of nicotine will further favour the induction of long-term potentiation. Besides causing long-term potentiation at glutamatergic synapses, nicotine also causes a long-lasting reduction of GABAergic input to principal neurons as a result of desensitisation of the nicotinic receptors present on these terminals (Mansvelder et al., 2002). So, whilst amphetamine blocks the induction of long-term depression, nicotine enables the induction of long-term potentiation (Mansvelder and McGehee, 2000).

A single in vivo injection of cocaine that induced behavioural sensitisation increases the AMPA/NMDA ratio (Ungless et al., 2001), which is also observed after the induction of long-term potentiation (Malinow and Malenka, 2001). The cocaine-induced increase in AMPA/NMDA ratio is NMDA receptor-dependent, indicating an involvement of long-term potentiation (Ungless et al., 2001). This is further confirmed by the fact that a single cocaine injection prevents the further induction of long-term potentiation and enhances the induction of long-term depression (Ungless et al., 2001). Taken together, these results indicate that a single injection of cocaine induced long-term potentiation of glutamatergic transmission onto principal neurons.

Is this effect specific for cocaine or does it also hold true for other drugs of abuse? Though cocaine, nicotine, morphine and ethanol have different loci of action and different underlying actions they have in common that they induce the same increase in AMPA/NMDA ratio after an in vivo exposure (Saal et al., 2003). Two non-addictive psychoactive compounds, fluoxetine and carbamazepine, did not produce this (Saal et al., 2003), indicating that the effect is specific for drugs of abuse. Interestingly, a stressful event also produced an increase in the AMPA/NMDA ratio, which fits in nicely with the fact that a stressful event can often lead to relapse to drugs of abuse (Saal et al., 2003).

So, in what way would long-term potentiation influence the output of the system? Enhanced glutamatergic transmission onto principal neurons will excite these neurons resulting in an increased dopamine outflow in the nucleus accumbens. In addition, increased excitation might lead to a facilitation of bursting activity, producing increased dopamine levels in the nucleus accumbens. The NMDA receptor is very important for this switch from regular to burst firing so enhancement of glutamatergic transmission would definitely favour this transition.

4. Modulation of cellular and synaptic activity in the ventral tegmental area

4.1. Catecholamines

4.1.1. Dopamine

In the ventral tegmental area, dopamine is released from dendrites of principal neurons after which it can affect the activity of surrounding principal neurons through somatodendritic and dendrodendritic contacts (Aghajanian and Bunney, 1977; Bayer and Pickel, 1990; Beart et al., 1979; Johnson and North, 1992b; Sesack et al., 1994).

4.1.1.1. Effects on principal neurons. Application of dopamine hyperpolarises principal neurons by activation of D2 receptors, causing an increase in a number of K^+ conductances, including I_K, I_A, and an anomalous rectifier (Liu et al., 1994; Momiyama et al., 1993). The action of dopamine on principal neurons is likely to be dendrodendritic as immunocytochemical studies show that D2 receptors are present on distal dendrites (Sesack et al., 1994) and dopamine is released from dendrites by the dopamine transporter (Beart et al., 1979; Falkenburger et al., 2001). Principal neurons projecting from the ventral tegmental area to cortical structures contain lower levels of autoreceptors and have higher firing frequencies (Chiodo et al., 1984). This indicates that within the ventral tegmental area subpopulations of principal cells exist with different levels of autoreceptors, resulting in different discharge rates and sensitivity to dopaminergic compounds (White and Wang, 1984a).

4.1.1.2. Effects on secondary neurons. One of the main criteria used to differentiate between principal and secondary neurons is their responsiveness to dopamine. Secondary neurons, thought to be GABAergic, do not respond electrophysiologically to dopamine (Johnson and North, 1992b).

4.1.1.3. Effects on synaptic transmission. The D1 receptor is present on GABAergic nerve terminals projecting from the nucleus accumbens to the ventral tegmental area (Mansour et al., 1991; Lu et al., 1997) Activation of this receptor enhances the release of GABA onto principal neurons (Cameron and Williams, 1993). This release of GABA increases the $GABA_B$ response on principal neurons whilst not affecting the $GABA_A$ response (Cameron and Williams, 1993). D1 receptors are also present on glutamatergic nerve terminals in the ventral tegmental area (Lu et al., 1998). Activation of these receptors enhances the release of glutamate onto principal neurons in the ventral tegmental area (Kalivas and Duffy, 1995). Whilst activation of D1 receptors enhances glutamate release, activation of D2 receptors

inhibits glutamatergic input onto principal neurons in the ventral tegmental area as a result of decrease Ca^{2+} influx into the nerve terminal (Koga and Momiyama, 2000). A regulation of GABA release by D2 receptors is unlikely as mRNA for D2 receptors was present in less than 3% of GABAergic neurons projecting to the ventral tegmental area from the nucleus accumbens, the main source of GABAergic input to the ventral tegmental area (Lu et al., 1998), though D2 receptors might be present on the presynaptic nerve terminals of local interneurons. Dopamine can therefore both increase and decrease the release of glutamate through different dopamine receptor subtypes. The release of GABA on the other hand can only be enhanced by dopamine, through activation of D1 receptors.

In summary, dopamine fulfils an auto-inhibitory role on its own activity by directly hyperpolarising the principal neuron through D2 receptors. Additionally, dopamine can affect the release of both glutamate and GABA. All drugs of abuse have in common that they increase accumbal levels of dopamine. This does however not automatically mean that all drugs of abuse increase the firing frequency or pattern of principal neurons. Cocaine, for instance, increases dopamine levels in the nucleus accumbens by blocking the re-uptake of dopamine. In the ventral tegmental area however this blockade of re-uptake will lead to increased dopamine levels, which will then act at the D2 receptor to inhibit the principal neuron.

4.1.2. 5-HT

From the dorsal raphe nuclei, where the cell bodies of the 5-HT system lie, a dense projection runs to the midbrain dopaminergic areas and these fibres form connections with both principal and secondary neurons (Herve et al., 1987). 5-HT receptors have been divided into three subclasses, 5-HT_1, 5-HT_2 and 5-HT_3 receptors and the ventral tegmental area is relatively rich in 5-HT receptors, in particular 5-HT_{1B} receptors (Pazos et al., 1985).

Based on in vivo experiments, it was hypothesised that serotonergic projections provide a tonic inhibitory control of principal neurons, mediated by 5-HT_{2C} receptors (Di Mascio et al., 1998; Gobert et al., 2000). The fact that 5-HT-selective reuptake inhibitors reduce the activity of principal neurons in the ventral tegmental area (Di Mascio et al., 1998) is in line with this hypothesis.

A number of different drugs of abuse, including cocaine, amphetamine (Jones and Kauer, 1999), methylenedioxymetamphetamine (MDMA, 'Ecstasy') (Obradovic et al., 1996) and ethanol (Brodie et al., 1995) have been shown to interact with 5-HT systems. Besides blocking the dopamine transporter cocaine also blocks the 5-HT transporter, thus increasing the level of 5-HT in the ventral tegmental area (Ritz et al., 1987).

Further evidence for an involvement of serotonergic systems in the reinforcing properties of cocaine is the fact that 5-HT_{1B} knockout mice have an increased likelihood to self-administer cocaine (Rocha et al., 1998).

4.1.2.1. Effects on principal neurons. The membrane potential of principal neurons either depolarised (46%), hyperpolarised (11%) or did not change after application of 5-HT (Pessia et al., 1994). The inward current, which caused the hyperpolarisation was mimicked by a 5-HT_{1A} receptor agonist and blocked by a 5-HT_{1A} receptor antagonist. These principal neurons that hyperpolarised in response to 5-HT might in fact be the tertiary neurons mentioned earlier (Cameron et al., 1997).

The outward depolarising current is mimicked by a 5-HT_2 receptor agonist (Pessia et al., 1994). It is not exactly clear which ionic conductance underlies this depolarisation. A decrease of an unidentified K^+ conductance or an increase in I_h have both been implicated in this effect (Pessia et al., 1994).

4.1.2.2. Effects on secondary neurons. Secondary cells either depolarised (30%), hyperpolarised (28%) or showed no change in response to 5-HT, indicating a heterogenous population of secondary neurons (Pessia et al., 1994). The depolarisation is most likely mediated by 5-HT_{2C} receptors as secondary neurons express this receptor and in vivo recordings showed that stimulation of this receptor by a selective agonist activated secondary neurons (Di Giovanni et al., 2001).

4.1.2.3. Effects on synaptic transmission. One of the earlier in vitro reports of 5-HT action in the ventral tegmental area was that 5-HT inhibited the $GABA_B$-mediated synaptic potential on principal neurons (Johnson et al., 1992a). This was shown to be the result of activation of presynaptic 5-HT_{1B} receptors, located on a subpopulation of GABAergic afferents to principal neurons (Sugita et al., 1992). The subsequent disinhibition of principal cells could account for the increase in accumbal dopamine levels seen after infusion of 5-HT into the ventral tegmental area (Guan and McBride, 1989).

Besides blocking the dopamine transporter cocaine also increases 5-HT levels by blocking the 5-HT transporter (Ritz et al., 1987). This will lead to a reduction of the $GABA_B$-mediated inhibitory post-synaptic potential and a subsequent disinhibition of the principal neuron (Cameron and Williams, 1994). The frequency of $GABA_A$ receptor-mediated responses in the majority of principal neurons was increased after application of 5-HT. This is likely to be a reflection 5-HT depolarising secondary neurons through 5-HT_{2C} receptors and increasing their firing rate (Pessia et al., 1994). In some cells, a decrease in inhibitory post-synaptic current frequency was seen, likely the result of 5-HT hyperpolarising certain secondary neurons through 5-HT_{1A} receptors (Pessia et al., 1994).

Recently, it was shown that amphetamine, which besides dopamine also increases the release of 5-HT, decreases excitatory transmission onto principal and secondary ventral tegmental area neurons (Jones and Kauer, 1999). This effect

was mediated by 5-HT receptors, not by dopamine receptors (Jones and Kauer, 1999).

The effects of 5-HT on cells in the ventral tegmental area are rather complex. The in vitro data indicate that the hypothesised tonic inhibitory action on principal neurons in the ventral tegmental area by 5-HT is most likely an indirect effect through GABAergic inhibition.

4.1.3. Acetylcholine

The ventral tegmental area receives cholinergic input from the tegmental pedunculopontine nucleus and the lateral dorsal tegmental nucleus (Woolf, 1991) and both muscarinic (Weiner et al., 1990) and nicotinic receptors are expressed in the ventral tegmental area (Azam et al., 2002; Clarke, 1993; Klink et al., 2001). Nicotinic receptors are ligand-gated ion channels that can be assembled from an array of different subunits resulting in a number of different receptors with distinct properties and pharmacology. In the ventral tegmental area the mRNA for $\alpha2-\alpha7$ and $\beta2-\beta4$ subunits are expressed by both principal and secondary neurons albeit in different quantities (Charpantier et al., 1998; Klink et al., 2001). Muscarinic receptors are G-protein coupled receptors and can be divided into five different subgroups, M_1-M_5, of which M_1, M_3 and M_5 are coupled to G_q and M_2 and M_4 are coupled to $G_{i/o}$ (Hulme et al., 1990). Both nicotinic and muscarinic receptors play an important role in regulating the output of the ventral tegmental area. Nicotine, acting on nicotinic receptors increases the release of dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988; Schilstrom et al., 1998b) as does an agonist for the muscarinic receptor (Gronier et al., 2000). Stimulation of the laterodorsal tegmental nucleus, one of the main sources of excitatory cholinergic input to the ventral tegmental area, produced a fast transient increase in dopamine levels in the nucleus accumbens, followed by a slower, prolonged component of increased dopamine levels in the nucleus accumbens. Whilst the nicotinic receptors seem to be most important in the initial fast response, muscarinic receptors are responsible for the prolonged response (Forster et al., 2002; Yeomans et al., 2001). Muscarinic receptors appear to be more important in mediating the rewarding effects of brain stimulation than nicotinic receptors (Yeomans and Baptista, 1997; Yeomans et al., 2000). Muscarinic and nicotinic receptors also mediate different types of reward, with the muscarinic receptors being more important for hypothalamus-mediated reward, like eating and drinking (Rada et al., 2000), and the nicotinic receptors for mediating the reinforcing properties of tobacco (Koob, 1992; Watkins et al., 2000).

4.1.3.1. Effects on principal neurons. Acetylcholine acts at both nicotinic and muscarinic receptors on principal neurons in the ventral tegmental area. Activation of, mainly non-$\alpha7$, nicotinic acetylcholine receptors depolarises principal neurons and increases their firing rate but these effects desensitise rapidly (Calabresi et al., 1989; Pidoplichko et al., 1997; Yin and French, 2000).

Muscarine also depolarises and increases the firing rate of principal neurons (Lacey et al., 1990), through muscarinic M1 or M5 receptors (Grillner and Mercuri, 2002; Lacey et al., 1990).

4.1.3.2. Effects on secondary neurons. Activation of nicotinic receptors increases the firing rate of secondary neurons, though to a lesser extent than in principal neurons (Yin and French, 2000). Secondary neurons also desensitise more rapidly to the effects of nicotine than principal neurons (Yin and French, 2000). The effects of agonists for muscarinic receptors on secondary neurons have to our knowledge not been examined.

4.1.3.3. Effects on synaptic transmission. The fact that antagonists for NMDA and AMPA receptors attenuate the increase in firing frequency of principal neurons by nicotine (Grillner and Mercuri, 2002; Grillner and Svensson, 2000) and block the accumbal dopamine release by nicotine (Schilstrom et al., 1998a), suggests that glutamatergic projections to the ventral tegmental area are involved in mediating the effects of nicotine. Indeed, nicotine enhances glutamatergic input onto principal neurons through activation of presynaptic $\alpha7$-containing nicotinic receptors (Mansvelder and McGehee, 2000). This enhanced glutamate release enables the induction of long-term potentiation by just post-synaptic depolarisation (Mansvelder and McGehee, 2000). At GABAergic synapses onto principal neurons, activation of nicotinic receptors at first enhances the release of GABA but this effect rapidly desensitises, changing to a long-lasting depression of GABAergic transmission (Mansvelder et al., 2002).

Both excitatory synaptic potentials and inhibitory potentials in dopaminergic neurons are depressed by activation of presynaptic muscarinic M_3 receptors (Grillner et al., 1999, 2000). The depression of glutamate release by activation of muscarinic receptors preferentially blocks the NMDA component of excitatory transmission (Zheng and Johnson, 2003b). Acetylcholine can therefore also regulate neurotransmitter release by acting on presynaptic muscarinic M_3 receptors.

4.1.4. Noradrenaline

The action of noradrenaline on ventral tegmental area neurons has not received much attention, with few recent findings. There is evidence for connections between noradrenergic terminals and principal neurons in the ventral tegmental area (Bayer and Pickel, 1990). In the ventral tegmental area, noradrenaline modulates the firing pattern of the principal neurons, making it more regular without affecting the firing rate (Grenhoff et al., 1993; Grenhoff and Svensson, 1989; Linner et al., 2001).

4.1.4.1. Effect on principal neurons. In vitro noradrenaline hyperpolarises more than half of principal neurons and depolarises one third (Grenhoff et al., 1995). The hyper-

polarising effect is blocked by a dopamine D2 receptor antagonist indicating an indirect effect. After blockade of D2 receptors, only the depolarisation remained in principal neurons. This is in line with earlier findings showing an effect of noradrenaline through D2 dopamine receptors (White and Wang, 1984b). The depolarising effect of noradrenaline is mediated by α_1-adrenoceptors (Grenhoff et al., 1995).

4.1.4.2. Effect on secondary neurons. Secondary neurons are also depolarised by an α-adrenergic agonist and the frequency of $GABA_A$ mediated input onto both principal and secondary neurons is increased by this agonist (Grenhoff et al., 1995).

In conclusion, noradrenaline, through acting on α_1-adrenoceptors, can modulate the activity of both principal and secondary neurons in the ventral tegmental area. The overall effect on the dopaminergic output of the ventral tegmental area is dominated by the modulation of the firing pattern, rather than the firing rate.

4.2. Amino acids

4.2.1. Glycine

Glycine is best known as the main inhibitory neurotransmitter not only in the spinal cord and brainstem. But also in the mesencephalic dopamine system glycine, glycine receptors and fibres containing glycine are present, though not at high levels (Rampon et al., 1996). The glycine receptor is a pentamer comprised of α and β subunits that upon activation opens a chloride channel, leading to chloride influx and subsequent hyperpolarisation of the neuron.

4.2.1.1. Effects on principal neurons. Sharp-electrode recordings showed that glycine hyperpolarised over 80% of principal neurons in the ventral tegmental area (Zheng and Johnson, 2001). This hyperpolarisation is blocked by the glycine-receptor antagonist strychnine and is the result of a chloride influx (Zheng and Johnson, 2001). A remainder of principal cells depolarised in response to glycine administration. This was however also associated with a decrease in firing frequency (Zheng and Johnson, 2001). The mechanism behind this depolarisation is unknown.

4.2.1.2. Effects on secondary neurons. In addition to hyperpolarising principal neurons, glycine also hyperpolarises secondary neurons in the ventral tegmental area by a similar strychnine-sensitive chloride-mediated mechanism (Zheng and Johnson, 2001).

In dissociated cells from the ventral tegmental area of neonatal rats, glycine receptor activation causes a depolarisation. This effect of glycine is modified by ethanol, which can either enhance (Ye et al., 2001a) or decrease the depolarisation (Ye et al., 2001b). This dual effect of ethanol might reflect effects on principal versus secondary neurons, or different effects in subpopulations of principal neurons. The findings are interesting for understanding the pathophysiology of foetal alcohol syndrome as during this neonatal period neurons are sensitive to the damaging effects of ethanol.

4.2.1.3. Effects on synaptic transmission. Glycine inhibits tetrodotoxin-sensitive spontaneous inhibitory post-synaptic potentials on principal neurons, which is likely to reflect the hyperpolarisation observed in secondary neurons (Zheng and Johnson, 2001).

The net outcome of glycine acting in the ventral tegmental area depends on the balance between the direct inhibitory effect on the principal neuron and the disinhibition of this neuron by inhibition of GABAergic neurons, combined with possible indirect effects through other brain regions and interactions with other neurotransmitter systems. Up until now, no reports have been made of the effect of glycine on dopamine release in target areas of the ventral tegmental area.

4.2.2. ATP/adenosine

ATP and its metabolite adenosine are released as co-transmitters and can act as neurotransmitters on P2 and P1 purinoceptors, respectively. The P2 purinoceptors can be divided into P2X (ligand-gated ion channel) and P2Y (G-protein coupled) receptors and the P1 purinoceptors can be divided into A1, A2 and A3 (all G-protein coupled). In the ventral tegmental area, P2 purinoceptor mRNA and protein are present (Kanjhan et al., 1999; Vulchanova et al., 1996). There is however debate about whether functional receptors are present on the somata of ventral tegmental area neurons or if they are only transported along the axon to the nerve terminals.

Both ATP and adenosine have been shown to modulate dopaminergic transmission with ATP increasing dopamine levels and adenosine reducing dopamine levels in the nucleus accumbens in vivo (Krugel et al., 2001a,b, 2003). This could be by modulating dopamine release at the nerve terminal in the nucleus accumbens, by altering the firing pattern of the ventral tegmental area neurons or by modulating the input to ventral tegmental area neurons. The effects of ATP in the ventral tegmental area are directly on the principal neurons whilst adenosine asserts its effect by modulating the input to these neurons.

4.2.2.1. Effects on principal neurons. ATP acts on P2X purinoceptors in dissociated principal ventral tegmental area neurons, causing membrane depolarisation and Ca^{2+} influx (Sorimachi et al., 2002). Perfusion of a P2 purinoceptor agonist increased dopamine levels in the nucleus accumbens, possibly reflecting an increased firing resulting from membrane depolarisation as observed in dissociated neurons (Sorimachi et al., 2002). The direct effects of adenosine on principal neurons have, to our knowledge, not been studied.

4.2.2.2. Effects on secondary neurons. To our knowledge, the effect of ATP and adenosine on secondary neurons in the ventral tegmental area has not been studied.

4.2.2.3. Effects on synaptic transmission. Adenosine is released from nerve terminals and can act to inhibit both GABAergic and glutamatergic neurotransmission (Bonci and Malenka, 1999; Wu et al., 1995). Adenosine, through presynaptic A1 receptors located on glutamatergic and GABAergic terminals, inhibits mainly the $GABA_B$ response whilst affecting the $GABA_A$ inhibitory post-synaptic current and glutamatergic excitatory post-synaptic current to a lesser extent (Bonci and Malenka, 1999; Wu et al., 1995). Later it was found that the NMDA component of the EPSC is mainly affected whilst the potency of adenosine to reduce the AMPA component was lower (Wu et al., 1999). This is hypothesised to be due to glutamate spillover resulting in low concentrations at other synapses where it will then act at NMDA receptors due to the higher affinity of glutamate for these receptors (Jonas and Sakmann, 1992; Patneau and Mayer, 1990). To our knowledge, no effect of ATP on the release of GABA or glutamate in the ventral tegmental area has been reported.

The overall effects of ATP and adenosine on dopaminergic transmission are in part at the level of the nucleus accumbens and in part in the ventral tegmental area. ATP increases dopamine release in the nucleus accumbens (Krugel et al., 1999, 2001b). In the ventral tegmental area, activation of P2 purinoceptors has been shown to lead to Ca^{2+} influx, probably as a result of membrane depolarisation (Sorimachi et al., 2002). The increased somatodendritic dopamine release caused by P2 purinoceptor activation will however provide an additional inhibition of principal neurons by dopamine. A P2 purinoceptor antagonist decreased basal dopamine levels indicating a tonic effect of endogenous ATP (Krugel et al., 2001a). The finding that local perfusion of an ATP analogue in the ventral tegmental area enhanced dopamine release in the nucleus accumbens indicates that ATP in the ventral tegmental area enhances the dopaminergic output (Krugel et al., 2001a). This is supported by the finding that P2 purinoceptor activation leads to increased locomotor activity in the open field (Krugel et al., 2001a).

The effects of adenosine on dopaminergic transmission are in the nucleus accumbens and in the ventral tegmental area. Adenosine microinfusion in the nucleus accumbens reduced dopamine levels and antagonists of the P1 purinoceptor increased dopamine levels (Krugel et al., 2003), opposite to the effect of ATP (Krugel et al., 2003). In the ventral tegmental area, adenosine modulates the synaptic input to principal neurons. It inhibits both the release of GABA and glutamate from nerve terminals. On principal neurons, the adenosine-induced inhibition of the $GABA_B$ response is more profound than the inhibition of the $GABA_A$ response or of excitatory responses. The finding that adenosine is more potent at inhibiting the NMDA response compared to the AMPA response together with the fact that NMDA receptors are important for burst firing of principal neurons means that adenosine should reduce burst firing (Wu et al., 1999). This has not been tested as yet.

An interesting aspect of adenosine-mediated inhibition of neurotransmission is its interaction with drugs of abuse and the effects of treatment with drugs of abuse. In the ventral tegmental area, adenosine is constantly synthesised and released resulting in an adenosine tone. In cocaine-treated animals, this adenosine tone is increased and this selectively inhibits the metabotropic glutamate receptor mediated inhibitory post-synaptic potential (Fiorillo and Williams, 2000). AMPA-mediated excitatory responses are inhibited in cocaine-treated animals, but this only becomes obvious when recorded in the presence of amphetamine, which enhances the adenosine tone (Fiorillo and Williams, 2000). No difference is seen in the modulation of the $GABA_B$ response when comparing saline- to cocaine-treated animals (Fiorillo and Williams, 2000). As a result, in cocaine-treated animals the inhibitory side of glutamatergic transmission is decreased resulting in an increased excitation of the principal neuron, and thus providing a possible mechanism for the phenomenon of induced drug craving in animals exposed to cocaine. The exact mechanism in which the increased adenosine tone specifically regulates metabotropic glutamate receptors whilst less affecting ionotropic glutamate receptors or the $GABA_B$ response is unknown.

Besides adenosine, a role for ATP in drug addiction has also been suggested as P2 purinoceptor agonists produce behavioural effects similar to amphetamine and cause behavioural sensitisation (Krugel et al., 2001a).

4.2.3. Histamine

Another amino acid neurotransmitter innervating the midbrain dopamine system is histamine, which is released from neurons projecting from the tuberomammillary nucleus of the hypothalamus (Panula et al., 1989). In the central nervous system, histamine has been implicated in a number of physiological functions including food intake, anxiety and arousal (Brown et al., 2001). Histamine can act on postsynaptic histamine H_1 receptors, which are positively coupled to phospholipase C, postsynaptic histamine H_2 receptors, which are positively coupled to adenylyl cyclase or on the presynaptic histamine H_3 autoreceptor, which is negatively coupled to adenylyl cyclase. A number of different findings indicate that histamine negatively influences dopaminergic neurotransmission. First of all, histamine increases the reinforcement threshold and suppresses self-stimulation (Cohn et al., 1973). Additionally, a protocol that enhances the histaminergic tone inhibits morphine-induced conditioned place preference (Suzuki et al., 1995). Furthermore, antagonists for the histamine H_1 receptor increase dopamine levels in the nucleus accumbens (Dringenberg et al., 1998) and have reinforcing properties (Masukawa et al., 1993).

4.2.3.1. Effects on principal neurons. Although the ventral tegmental area receives histaminergic innervation, very little is known about its effect on principal neurons. The effect of histamine on identified principal neurons in the ventral tegmental area has been studied with in vitro extracellular recordings and no effect on the firing frequency was found (Korotkova et al., 2003).

4.2.3.2. Effects on secondary neurons. The same study showed that histamine increases the firing frequency of GABAergic neurons in the ventral tegmental area through activation of histamine H_1 receptors (Korotkova et al., 2003).

4.2.3.3. Effects on synaptic transmission. To our knowledge, no reports of histamine action on synaptic transmission in the ventral tegmental area have been made.

The finding that histamine increases the firing frequency of GABAergic neurons whilst not affecting principal neurons is in line with the idea that brain histaminergic systems functionally antagonise dopaminergic systems. An increase in GABAergic cell firing will lead to an enhanced inhibition of the principal neuron and subsequent dopaminergic output.

As mentioned before, histamine was shown to negatively influence drug reinforcement, decrease self-stimulation and block morphine-induced conditioned place preference (Cohn et al., 1973; Suzuki et al., 1995). The described electrophysiological actions of histamine in the ventral tegmental area, through secondary neurons, provide a mechanism by which histamine can mediate these effects.

4.3. Neuropeptides

4.3.1. Opioids

The opioid class of receptors comprises of the μ, δ- and κ-opioid receptors. A number of endogenous opioids, like endorphins and enkephalins as well as exogenous compounds, like heroin and morphine can act at these receptors, with different affinities. All three opioid-receptor subtypes are G-protein coupled and act to inhibit adenylyl cyclase (Knapp et al., 1995). In situ hybridisation and immunohistochemical studies show that all three receptor subtypes are present throughout the mesolimbic system (Mansour et al., 1994, 1995, 1996). Opioid transmitter systems have been implicated in a number of physiological processes, most notably nociception (Mao, 1999; Markenson, 1996) and the physiology of reward (Van Ree et al., 2000). Microinjections of agonists for the μ- and δ-receptor increased dopamine levels in the ventral striatum with the μ-opioid receptor agonist being most potent (Devine et al., 1993). An agonist for the κ-receptor produced no increase in ventral striatal dopamine levels when injected locally in the ventral tegmental area, though systemic administration of this compound did cause increased levels (Devine et al., 1993). Morphine and selective agonists for the μ- and δ-opioid receptor are self-administered into the ventral tegmental area of rats by lever pressing (Devine and Wise, 1994). The μ-receptor agonist was more effective, indicating a prominent role for this receptor (Devine and Wise, 1994). Furthermore, activation of μ-opioid receptors in the ventral tegmental area produced conditioned place preference whilst activation of the κ-opioid receptor in the ventral tegmental area produced conditioned place aversion (Bals-Kubik et al., 1993; McBride et al., 1999). Overall, these biochemical and behavioural data suggest that activation of the μ-opioid receptor causes reinforcement, as does activation of the δ-opioid receptor. Of these two, the μ-opioid receptor seems to play the most important role in mediating the reinforcing properties of morphine. Activation of the κ-opioid receptor on the other hand has an aversive effect.

4.3.1.1. Effects on principal neurons. Morphine increases the firing rate of principal neurons in both the ventral tegmental area and substantia nigra when measured extracellularly in vivo (Gysling and Wang, 1983). This is thought to be an indirect effect by reducing the activity of secondary neurons in the vicinity (Gysling and Wang, 1983). As mentioned, one of the main criteria for the division of ventral tegmental area neurons into subgroups was the responsiveness to opioids. Principal neurons do not respond whereas secondary neurons hyperpolarise in response to opioid peptides (Johnson and North, 1992b). The increase in principal cell firing observed in vivo is most likely the result of disinhibition due to secondary neuron hyperpolarisation by activation of μ-opioid receptors present on these neurons (Johnson and North, 1992a,b).

4.3.1.2. Effect on secondary neurons. Secondary neurons are hyperpolarised by opioid peptides. This hyperpolarisation is mediated by the μ-opioid receptor, with no effect of δ- and κ-receptor agonists (Johnson and North, 1992a,b).

4.3.1.3. Effects on synaptic transmission. Activation of μ-opioid receptors decreases the frequency of spontaneous inhibitory post-synaptic currents as a result of hyperpolarisation of secondary neurons (Johnson and North, 1992a). Recently, electron microscopy and recordings from cultured neurons have provided evidence for an additional presynaptic μ-opioid receptor that regulates GABAergic input to principal neurons (Bergevin et al., 2002; Garzon and Pickel, 2002; Svingos et al., 2001). The existence of a presynaptic μ-opioid receptor that regulates GABAergic and glutamatergic transmission has already been shown in a number of other brain areas including hippocampus (Cohen et al., 1992), globus pallidus (Stanford and Cooper, 1999), subthalamic nucleus (Shen and Johnson, 2002), dorsal horn of the spinal cord (Kerchner and Zhuo, 2002) and substantia gelatinosa (Grudt and Henderson, 1998).

Electron microscopical studies also showed the presence of μ-opioid receptors on the distal dendrites of a small number of tyrosine hydroxylase-positive neurons (Svingos

et al., 2001). These neurons might correspond to the tertiary cells as approximately one third of tertiary neurons are tyrosine hydroxylase-positive and these neurons are [Met^5]-enkephalin responsive (Cameron et al., 1997).

Taken together, activation of the μ-opioid receptor leads to a disinhibition of principal neurons by inhibiting secondary neurons. The role of the δ- and κ-opioid receptors and the importance of tertiary neurons will require more investigation.

4.3.2. Nociceptin/orphanin FQ

Following the cloning of the opioid receptors, screening of cDNA libraries revealed a related receptor, later to be termed opioid-receptor like receptor 1 (ORL1) (Mollereau et al., 1994). This receptor showed a high structural homology with the 'classic' opioid receptors, but the known endogenous opioids had little or no effect at these receptors, thus rendering it an orphan opioid receptor. A year after the discovery of the orphan opioid receptor the endogenous agonist was found, termed nociceptin or orphanin/FQ (Reinscheid et al., 1995). mRNA for the ORL1 receptor is found in a number of tissues and shows a wide distribution in the rat and mouse brain (Anton et al., 1996). In the ventral tegmental area, mRNA expression for the ORL1 receptor is found in principal neurons whilst mRNA for nociceptin/orphanin FQ is found in the secondary neurons (Norton et al., 2002).

The ORL1 receptor is a $G_{i/o}$-protein coupled receptor, inhibiting adenylate cyclase. ORL1 activation enhances an inwardly rectifying K^+ conductance in a number of brain areas, including locus coeruleus (Connor et al., 1996) and hippocampus (Madamba et al., 1999). Additionally, nociceptin/orphanin FQ inhibits voltage-dependent Ca^{2+} channels, important for neurotransmitter release, in the hippocampus (Knoflach et al., 1996). This provides nociceptin/orphanin FQ with a whole repertoire of possible actions, both at the cellular and synaptic level. Physiological processes, in which the ORL1 receptor has been implicated include stress (Devine et al., 2001), anxiety (Jenck et al., 1997), food intake (Pomonis et al., 1996), nociception (Reinscheid et al., 1995), motor co-ordination (Devine et al., 1996a,b), locomotion (Florin et al., 1996) and learning and memory (Sandin et al., 1997).

The overall effect of nociceptin/orphanin FQ on the mesolimbic dopamine system is suppression of the dopaminergic output to the nucleus accumbens. At relatively high doses, nociceptin/orphanin FQ suppresses the release of dopamine in the nucleus accumbens (Murphy et al., 1996), but nociceptin/orphanin FQ by itself produces no conditioned place preference or aversion (Devine et al., 1996a,b). Nociceptin/Orphanin FQ, however, blocks the acquisition of place preference by morphine (Murphy et al., 1999). Furthermore, although nociceptin/orphanin FQ at low doses does not affect accumbal dopamine levels it does block the morphine-induced increase (Di Giannuario et al., 1999; Di Giannuario and Pieretti, 2000). Whether or not these effects on the reinforcing properties of morphine are relevant for other drugs of abuse remains to be answered.

4.3.2.1. Effects on principal neurons. Sharp-electrode recordings showed a direct, tetrodotoxin and naloxone-insensitive, hyperpolarisation of ventral tegmental area principal neurons, which reversed close to the K^+ equilibrium constant (Zheng et al., 2002). This is consistent with findings in other brain areas that nociceptin/orphanin FQ directly hyperpolarises neurons by activating inwardly rectifying K^+ channels (Connor et al., 1996; Madamba et al., 1999).

4.3.2.2. Effects on secondary neurons. Nociceptin/orphanin FQ also hyperpolarises most secondary neurons, again with a reversal potential close to the expected K^+ equilibrium constant (Zheng et al., 2002). This is somewhat surprising as no ORL1 receptor mRNA was found on secondary neurons in the ventral tegmental area (Norton et al., 2002).

4.3.2.3. Effects on synaptic transmission. Nociceptin/orphanin FQ reduced the frequency of tetrodotoxin-sensitive spontaneous inhibitory post-synaptic potentials whilst leaving the amplitude unaffected (Zheng et al., 2002). As nociceptin/orphanin FQ also significantly increased the paired-pulse ratio of evoked inhibitory post-synaptic potentials, it appears to have a presynaptic locus of action where it can further inhibit the GABAergic input onto principal neurons (Zheng et al., 2002). Nociceptin/orphanin FQ inhibits both GABAergic and glutamatergic transmission in the periaqueductal gray (Vaughan et al., 1997). The effect of nociceptin/orphanin FQ on glutamatergic transmission in the ventral tegmental area has to our knowledge not been investigated yet.

Nociceptin/orphanin FQ by itself does not produce an effect on behavioural reinforcement or on dopamine levels in the nucleus accumbens. It does however attenuate morphine-induced responses. As nociceptin/orphanin FQ acts on both principal and secondary neurons in the ventral tegmental area, an explanation for this might be that under resting conditions these two effects are in balance, whilst the inhibitory component is strong enough to block the effects of morphine.

4.3.3. Orexins

Orexins are another neurotransmitter group that has recently been shown to have an effect on the excitability of cells in the ventral tegmental area. The ventral tegmental area receives a large amount of input from the lateral hypothalamus including cells containing the neurotransmitters orexin A and B (Fadel and Deutch, 2002). The hypothalamus is involved in the regulation of energy balance. Besides affecting the feeding pattern, orexins are also involved in locomotor activity and arousal (Willie et al., 2001). The ventral tegmental area is involved in the reaction

to primary rewards, like food, and the subsequent arousal and locomotor activity to obtain the reward (Schultz, 1998). Therefore, the connection between the hypothalamus and the ventral tegmental area may be important in the effects of orexins. Besides being important in mediating the response to primary rewards, a role for orexins was described recently in morphine dependence and withdrawal as orexin knock-out mice show attenuated morphine dependence (Georgescu et al., 2003).

4.3.3.1. Effects on principal neurons. Application of orexin A results in burst-like behaviour, a slight, tetrodotoxin-independent, depolarisation and an increase in firing rate, or no change in membrane potential (Korotkova et al., 2003). Interestingly, the cells where the burst-like firing was induced have a relatively small slow afterhyperpolarisation. This correlates nicely with the finding that blockade of the slow afterhyperpolarising current by apamin aids in the induction of burst firing by nickel or NMDA (Seutin et al., 1993). Orexin B also increased the firing rate to a similar extent as orexin A in most of the cells that responded to orexin A and orexin B was also able to induce the burst-like firing in a number of cells (Korotkova et al., 2003).

4.3.3.2. Effects on secondary neurons. Secondary neurons respond to orexins with an increase in firing rate and slight depolarisation. The increase in firing rate was particularly large in cells with a slow initial firing rate (Korotkova et al., 2003).

4.3.3.3. Effects on synaptic transmission. No studies have been reported yet on the effects of orexins on presynaptic GABA and glutamate release in the ventral tegmental area.

4.4. Endocannabinoids

Endocannabinoids act as retrograde messengers in a number of different brain areas, including the hippocampus (Wilson and Nicoll, 2001), the cerebellum (Kreitzer and Regehr, 2001a,b; Wilson and Nicoll, 2001), the amygdala (Katona et al., 2001) and the neocortex (Trettel and Levine, 2003). The cannabinoid receptor that is present in the brain, the cannabinoid CB_1 receptor, is widely distributed throughout the brain (Moldrich and Wenger, 2000), though no cannabinoid CB_1 receptor mRNA is present in the adult midbrain (Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998). As endocannabinoids act as retrograde messengers, cannabinoid receptors present on presynaptic terminals from other brain areas might however play an important role in regulating the activity of neurons in the ventral tegmental area. Besides being important in mediating the effects of endocannabinoids, the cannabinoid CB_1 receptor is also the target for the active ingredient of the recreational drug marijuana, so the role of cannabinoid CB_1 receptors in mesolimbic structures like the nucleus accumbens and ventral tegmental area is of particular interest.

4.4.1. Effects on principal neurons

In vivo extracellular recordings showed that the active ingredient of marijuana, delta9-tetrahydrocannabinol, excites rat ventral tegmental area neurons through cannabinoid CB_1 receptors (French, 1997; French et al., 1997). As cannabinoid CB_1 receptor mRNA is not present in the adult midbrain, it is unlikely that this represents a direct effect (Mailleux and Vanderhaeghen, 1992). One interesting finding of the study is that a selective cannabinoid CB_1 receptor antagonist, SR141716A, produces a significant decrease in the firing rate of the principal neurons indicating a tonic activation of cannabinoid CB_1 receptors by endocannabinoids (French, 1997). This effect of the cannabinoid CB_1 receptor antagonist was however not replicated in other in vivo studies (Cheer et al., 2003; Gessa et al., 1998). These contradicting results may be due to the complex, and not fully understood, pharmacology of this compound, including reports of inverse agonistic properties (Landsman et al., 1997). Additionally, in slice preparations indirect effects on firing frequency might be lost due to loss of synaptic inputs. Thus, the question of tonic activation of the receptor by endocannabinoids remains as yet unanswered.

4.4.2. Effects on secondary neurons

What the direct effect of cannabinoids is on the excitability of secondary cells has, to our knowledge, never been tested.

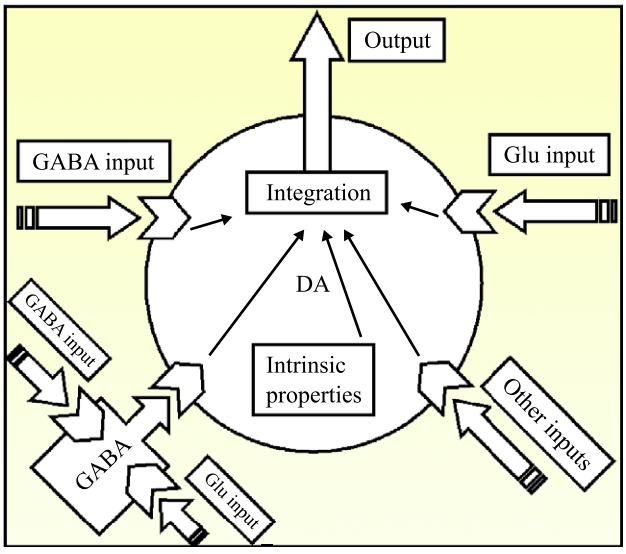

Fig. 2. Integration of input and intrinsic properties by the principal neuron in the ventral tegmental area. The principal, dopaminergic, neuron receives direct glutamatergic and GABAergic innervation. In addition, other neurotransmitters directly innervate the principal neuron. Furthermore, input to the secondary, GABAergic, neurons in the ventral tegmental area indirectly influences the principal neuron. The input and intrinsic properties of the neuron are integrated to establish the firing pattern and frequency, which determines the dopaminergic output from the ventral tegmental area.

4.4.3. Effects on synaptic transmission

Principal and secondary neurons in the ventral tegmental area receive GABAergic input from medium spiny neurons in the nucleus accumbens and from interneurons in the ventral tegmental area. WIN55212-2, a mixed cannabinoid CB1/CB2 receptor agonist inhibited evoked inhibitory post-synaptic currents onto both principal and secondary neurons (Szabo et al., 2002). This effect was attenuated by the cannabinoid CB_1 receptor antagonist SR141716A (Szabo et al., 2002). The results indicate a presynaptic mechanism, as expected. Whether the presynaptic cannabinoid CB_1 receptor receptors are present on GABAergic interneurons or on GABAergic projection neurons from other neurons, e.g. medium spiny neurons from the nucleus accumbens, remains to be determined. The absence of mRNA for the cannabinoid CB_1 receptor in adult midbrain regions however indicates that GABAergic projection neurons from the nucleus accumbens to the ventral tegmental area are the main target for cannabinoids.

No reports on the effects of cannabinoids on excitatory transmission in the ventral tegmental area have been made yet. It has however been shown that excitatory transmission onto substantia nigra pars compacta neurons is inhibited by cannabinoids (Szabo et al., 2000).

In summary, cannabinoids excite principal neurons in the ventral tegmental area and this excitation is likely to be the result of decreased GABAergic input onto these neurons. This excitation of principal neurons explains the increase in dopamine release observed in the nucleus accumbens (Tanda et al., 1997), and ventral tegmental area (Chen et al., 1993) after cannabinoid application. The increase in dopamine levels in the nucleus accumbens in turn accounts for the reinforcing properties of cannabinoids. Delta9-tetrahydrocannabinol, the active ingredient of marijuana-induced conditioned place preference (Braida et al., 2001b), is self-administered (Fattore et al., 2001; Martellotta et al., 1998) and lowers the threshold for electrical self-stimulation (Gardner et al., 1988).

Whether or not opioid systems are involved in mediating the effects of cannabinoids is still a matter of debate. Some groups have argued in favour of a role of opioids (Braida et al., 2001a; Chen et al., 1990; Navarro et al., 2001; Tanda et al., 1997), whilst others provide evidence against involvement of opioid systems (French, 1997; Melis et al., 2000; Welch, 1993).

Table 1
In vitro data on modulation of cell excitability and synaptic transmission by excitatory, inhibitory inputs and neuromodulatory neurotransmitters

Neurotransmitter	Receptor	Principal neurons	Secondary neurons	Effect on GABAergic input to principal neuron	Glutamatergic input to principal neuron
Glutamate	NMDA	+	+		
	AMPA	+	+		
	mGluRs	+/−	+		−[1]
GABA	$GABA_A$	−	−		
	$GABA_B$	−	−		−[1]
Dopamine	D1		n.e.	+[2]	+
	D2	−	n.e.		−
Serotonin	$5\text{-}HT_{1A}$	−	−	−[3]	
	$5\text{-}HT_{1B}$			−[2]	−?
	5-HT2	+	+	+[3]	
Acetylcholine	nAChR	+[4]	+[4]	+[4]	+
	mAChR	+		−	−[1]
Noradrenaline	α1-AR	+/(−)	+	+[3]	
Glycine	GlyR	−/(+)	−	−[3]	
ATP	P2	+			
Adenosine	P1			−[2]	−[1]
Histamine	H1	n.e.	+		
Opioid	Mu	n.e.	−	−[3]	
	Delta		n.e.	n.e.	
	Kappa		n.e.	n.e.	
Nociceptin/orphanin FQ	ORL-1	−	−	−	
Orexin A		+	+		
Orexin B		+	+		
Endocannabinoids	CB1			−	

+ = positive effect (depolarisation and/or increased firing frequency and/or increased glutamate/GABA release).
− = negative effect (hyperpolarisation and/or decreased firing frequency and/or decreased glutamate/GABA release).
n.e. = no effect.
open = not determined.
1 = mainly on NMDA component.
2 = mainly on $GABA_B$ component.
3 = mainly on $GABA_A$ component, can be result of effect on secondary neuron.
4 = desensitises rapidly.

The cannabinoid system has also been implicated in affecting the reinforcing properties of other drugs of abuse, including cocaine (Fattore et al., 1999), 3,4-methylenedioxymethamphetamine (MDMA, 'Ecstasy') (Braida and Sala, 2002) and alcohol (Poncelet et al., 2003; Racz et al., 2003). Furthermore, cannabinoids have been shown to provoke relapse to cocaine after withdrawal and cannabinoid receptor antagonists have therapeutic potential in preventing relapse (De Vries et al., 2001).

5. Concluding remarks

The activity of the main output of the mesolimbic dopamine system, namely the dopaminergic output from principal neurons in the ventral tegmental area projecting to the nucleus accumbens, depends on a number of factors. First of all, excitatory and inhibitory input onto these principal neurons influence their activity. These inputs can be regulated by neuromodulatory neurotransmitters, which can also directly affect the activity of the principal neurons. Furthermore, inhibitory input from local secondary interneurons will affect the activity of principal neurons. These secondary neurons in turn also receive excitatory and inhibitory input that can be regulated. Neuromodulatory neurotransmitters can also affect the activity of secondary neurons and thus indirectly influence the activity of principal neurons. Taken together the sum of inhibitory and excitatory input to the principal neuron, combined with the intrinsic properties, regulates the output of the principal neuron (Fig. 2). The effect of modulatory neurotransmitters depends on what component of the ventral tegmental area is affected (see Table 1).

Another aspect of neurotransmission that modulates the output of the ventral tegmental area is activity-dependent synaptic plasticity, which is sensitive to drugs of abuse. Drugs of abuse induce long-term potentiation of glutamatergic input onto principal cells in the ventral tegmental area, thereby increasing the output of the ventral tegmental area. In conclusion, the reviewed in vitro data can explain some of the results from in vivo experiments by showing that neurotransmitter systems affect certain targets in the ventral tegmental area. Some of the neurotransmitters have multiple actions on cells and neurotransmission in the ventral tegmental area, which may account for some of the paradoxical results found in vivo. In addition, these data can be used to guide future research in which specific modulation of a specific target can be investigated.

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