Progress in Neuro-sychopharmacology & Biological Psychiatry 32 (2008) 1399–1419

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Review article

The effects of intra-cerebral drug infusions on animals' unconditioned fear reactions: A systematic review

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ARTICLE INFO

Article history:
Received 1 August 2007
Received in revised form 25 March 2008
Accepted 26 March 2008
Available online 8 April 2008

Keywords:
Amygdala
Anxiety
Gamma aminobutyric acid
Neuropeptide
Septum
Serotonin

ABSTRACT

Intra-cerebral (i.c.) microinfusion of selective receptor agonists and antagonists into behaving animals can provide both neuroanatomical and neurochemical insights into the neural mechanisms of anxiety. However, there have been no systematic reviews of the results of this experimental approach that include both a range of unconditioned anxiety reactions and a sufficiently broad theoretical context. Here we focus on amino acid, monoamine, cholinergic and peptidergic receptor ligands microinfused into neural structures previously implicated in anxiety, and subsequent behavioral effects in animal models of unconditioned anxiety or fear. $GABA_A$ receptor agonists and glutamate receptor antagonists produced the most robust anxiolytic-like behavioral effects, in the majority of neural substrates and animal models. In contrast, ligands of the other receptor systems had more selective, site-specific anti-anxiety effects. For example, 5-HT_{1A} receptor agonists produced anxiolytic-like effects in the raphe nuclei, but inconsistent effects in the amygdala, septum, and hippocampus. Conversely, 5-HT_3 receptor antagonists produced anxiolytic-like effects in the amygdala but not in the raphe nuclei. Nicotinic receptor agonists produced anxiolytic-like effects in the raphe and anxiogenic effects in the septum and hippocampus. Unexpectedly, physostigmine, a general cholinergic agonist, produced anxiolytic-like effects in the hippocampus. Neuropeptide receptors, although they are popular targets for the development of selective anxiolytic agents, had the least reliable effects across different animal models and brain structures, perhaps due in part to the fact that selective receptor ligands are relatively scarce. While some inconsistencies in the microinfusion data can easily be attributed to pharmacological variables such as dose or ligand selectivity, in other instances pharmacological explanations are more difficult to invoke: e.g., even the same dose of a known anxiolytic compound (midazolam) with a known mechanism of action (the benzodiazepine–$GABA_A$ receptor complex), can selectively affect different fear reactions depending upon the different subregions of the nucleus into which it is infused (CeA versus BLA). These particular functional dissociations are important and may depend on the ability of a $GABA_A$ receptor agonist to interact with distinct isoforms and combinations of $GABA_A$ receptor subunits (e.g., α1-6, β1-3, ϒ1-2, δ), many of which are unevenly distributed throughout the brain. Although this molecular hypothesis awaits thorough evaluation, the microinfusion data overall give some support for a model of "anxiety" that is functionally segregated along different levels of a neural hierarchy, analogous in some ways to the organization of sensorimotor systems.

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Abbreviations: 5-HT, 5-hydroxytryptamine; BLA, basolateral amygdala; CeA, central amygdala; CRF, corticotrophin releasing factor; dH, dorsal hippocampus; DRN, dorsal raphe nucleus; EPM, elevated plus-maze; GABA, gamma amino butyric acid; HPA, hypothalamic pituitary adrenal axis; i.c., intra-cerebral; i.c.v., intracerebroventricular; LD, light/dark exploration test; LS, lateral septum; MeA, medial amygdale; mPFC, medial prefrontal cortex; MRN, median raphe nucleus; MS, medial septum; NPY, neuropeptide Y; PAG, periaqueductal gray; SPA, shock-probe avoidance; SPB, shock-probe burying; USV, ultrasonic vocalization; vH, ventral hippocampus.

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doi:10.1016/j.pnpbp.2008.03.020

1. Introduction

Over the past four decades, animal indices of "fear" or "anxiety"[1] have been used as simple "screening tests" of potential anxiolytic compounds, and as "models" to study the neural mechanisms of anxiety and anxiolytic drug action, (Bourin and Hascoet, 2003; Griebel, 1995; Treit, 1985). Three criteria distinguish simple screening tests from models specifically used to study the neural bases of anxiety: 1) correspondence in form between the expression of fear in the animal model and its expression in humans 2) continuity of function between fear in animals and anxiety in humans, and 3) conservation of the underlying brain mechanisms of fear and anxiety across mammalian species. While these criteria are theoretical imperatives for animal models of anxiety, in practice they are difficult to satisfy unambiguously (Treit, 1985). The very best animal models of anxiety are incomplete approximations of the human condition. The hope is that some aspect of the model will ultimately relate to the behavioral and neural correlates of 'anxiety' in humans, normal or pathological.

There is a vast literature in which animal fear reactions have been used to study the effects of peripherally administered anxiolytic drugs (for reviews, see File and Seth, 2003; Graeff, 2002; Griebel, 1995; Igor et al., 2001; Rodgers, 1997; Treit, 1985).

While these studies have provided a wealth of information about the behavioral effects of anxiolytic drugs (e.g., Valium®, BuSpar®), the widespread distribution of these drugs after peripheral administration obscures their site-specific effects in the brain. Another, more direct approach for studying the neural mechanisms of anxiety is to lesion selected brain structures and/or neurotransmitter systems and to examine the effects on animal fear reactions (e.g., File et al., 1979; Shah and Treit, 2003). The specificity of brain lesioning techniques, however, is often inadequate for the unambiguous assessment of brain function. Even relatively specific lesions of cell bodies, axons, or neurotransmitter systems provide limited information about the function of specific receptor populations within the denervated brain area. This is particularly important given that the function of well-defined receptor systems (e.g., $GABA_A$) can vary across subregions of a single, neuroanatomically defined structure (Kaufmann et al., 2003). Intra-cerebroventricular (ICV) administration of specific receptor agonists or antagonists provides more detailed neurochemical information, but the anatomical specificity of this technique is weak. Most compounds are distributed more or less evenly throughout the brain after ICV administration.

A third technique—site-specific intra-cerebral (i.c.) microinfusion of selective receptor agonists or antagonists—seems to combine the utility of other techniques used to stimulate or inhibit brain function, with a physiologically more selective and subtle effect. Although not without its own complexities and pitfalls (see Greenshaw, 1998; Menard and Treit, 1999), in principle i.c. infusion techniques can provide detailed information about both the anatomical and neurochemical substrates of anxiety, as expressed in animal models.

Although a very large body of empirical data is summarized in this review, it will become apparent to the reader that much of it has been derived from a small number of 'limbic' structures (e.g., amygdala) and neurotransmitter systems (e.g., GABA). This restricted data base ought not to constrain our view of the neural mechanisms of anxiety, however. Indeed, there is evidence, presented in this review, that many different structures and transmitter molecules contribute the behavioral expressions of anxiety in animal models (see Tables 1–8). Theoretical models of the neurobiological basis of anxiety must ultimately be able to accommodate this wealth of empirical data; otherwise, these models will be as tenuous as they are incomplete.

Thus, the major purpose of this review is to give readers a systematic and inclusive description of existing findings. A secondary purpose is to discuss, with appropriate caution, some of the theoretical implications of the data. Finally, it should be noted that while these microinfusion data may relate to the external expressions of anxiety in humans and other animals, they do not necessarily illuminate its natural causes or etiology. With these caveats in mind, we can turn our attention to a rationale for our focus on specific animal models.

Most animal models have been designed to represent either conditioned (e.g., Geller conflict paradigms) or unconditioned anxiety (e.g., elevated plus-maze; for reviews see Treit, 1985; Treit et al., 2003). Because animal models of unconditioned fear or anxiety do not explicitly require learning or memory, the effects of neuropharmacological interventions on anxiety-related behaviors in these models can be more easily separated from effects on more complex, cognitive processes. Thus, a combination of i.c. infusion techniques and "ethologically-inspired" behavioral techniques may provide relatively specific and unique neuroanatomical and neurochemical insights into the neural mechanisms of anxiety.

Accordingly, this review will focus on the behavioral effects of intra-cerebral microinfusions of selective agonists and/or antagonists, in five widely used models that are explicitly based on animals' untrained defense reactions: the elevated plus-maze test [EPM], shock-probe burying test [SPB], light/dark exploration test [LD], social interaction test [SI], and the separation-or shock-induced ultrasonic vocalization test [USV]. The studies included in this review are based on a search of the ISI Web of Science for articles published between 1970 and 2007, using model names, neuroanatomical locations (see below), neurotransmitters, and receptor subtypes as search keywords, each combined with the terms "anxiety" or "fear."

1.1. Organization of the review

We have organized the empirical findings in this review anatomically, along a caudal–rostral axis, from the brainstem (locus coeruleus, raphe nuclei, periaqueductal gray) to the forebrain (hypothalamus, amygdala, septum, hippocampus, medial prefrontal cortex), mainly for convenience. Nonetheless, all of the structures in this neural hierarchy are interconnected, and have been implicated in fear and anxiety using complementary methodologies such as Fos immunohistochemistry, receptor knockout, electrophysiology, and functional brain imaging (for reviews see Singewald et al., 2003; Singewald and Sharp, 2000; Finn et al., 2003; Linden 2006; Ressler and Mayberg, 2007; Davidson, 2002). The neuroanatomy, neurochemistry and receptor distributions of each of these target structures has been reviewed elsewhere (e.g., Paxinos, 1995), and only brief summaries of

[1] To be consistent with much of the animal literature, we use the terms "anxiety" and "fear" interchangeably, although some researchers have made distinctions between these two terms based on theoretical and behavioral criteria (e.g., Blanchard and Blanchard, 1990). In both instances, however, our exact focus is on "unconditioned" fear or anxiety.