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MONOAMINES TISSUE CONTENT ANALYSIS REVEALS RESTRICTED AND SITE-SPECIFIC CORRELATIONS IN BRAIN REGIONS INVOLVED IN COGNITION

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Abstract—The dopamine (DA), noradrenalin (NA) and serotonin (5-HT) monoaminergic systems are deeply involved in cognitive processes via their influence on cortical and subcortical regions. The widespread distribution of these monoaminergic networks is one of the main difficulties in analyzing their functions and interactions. To address this complexity, we assessed whether inter-individual differences in monoamine tissue contents of various brain areas could provide information about their functional relationships. We used a sensitive biochemical approach to map endogenous monoamine tissue content in 20 rat brain areas involved in cognition, including 10 cortical areas and examined correlations within and between the monoaminergic systems. Whereas DA content and its respective metabolite largely varied across brain regions, the NA and 5-HT contents were relatively homogenous. As expected, the tissue content varied among individuals. Our analyses revealed a few specific relationships (10%) between the tissue content of each monoamine in paired brain regions and even between monoamines in paired brain regions. The tissue contents of NA, 5-HT and DA were inter-correlated with a high incidence when looking at a specific brain region. Most correlations found between cortical areas were positive while some cortico-subcortical relationships regarding the DA, NA and 5-HT tissue contents were negative, in particular for DA content. In conclusion, this work provides a useful database of the monoamine tissue content in numerous brain regions. It suggests that the regulation of these neuromodulatory systems is achieved mainly at the terminals, and that each of these systems contributes to the regulation of the other two.

Key words: monoamine tissue content, serotonin, dopamine, noradrenalin, cognition, rat.

INTRODUCTION

Cognitive information processing involves a crosstalk between numerous cortical and subcortical regions whose interactions are modulated by the dopamine (DA), noradrenalin (NA) and serotonin (5-HT) monoaminergic systems (Dalley et al., 2011; Homberg, 2012). Each monoamine system shapes a broad variety of behavioral and cognitive traits such as attention, impulsivity or reward seeking, through inter-individual differences in monoaminergic activities and interactions (Dalley et al., 2011). Imbalance or dysfunction in these systems has been implicated in numerous neuropsychiatric diseases including schizophrenia, depression, drug abuse, Parkinson's disease, obsessive compulsive disorder and attentional deficit hyperactivity disorder (Soubrie et al., 1984; Koob, 1992; Nieoullon, 2002; Millan, 2006; Delaville et al., 2012; Navailles and De Deurwaerdère, 2012). The widespread nature of these monoaminergic networks is one of the main difficulties in understanding their functions and interactions in normal and pathological states.

One general feature of monoaminergic neurons is the high density of their axonal arbor that can target multiple brain areas and establish millions of synaptic or "en-passant" contacts per mm^2 of target regions. Interestingly, these dense axonal fields originate from a relatively few number of cell bodies that are confined to the substantia nigra pars compacta (SNc), the retrorubral area and ventral tegmental area (VTA) for DA neurons (Björklund and Lindvall, 1986; Gerfen, 1992), the A1/A2 regions and the locus coeruleus (LC) for NA neurons (Aston-Jones, 2004) and the dorsal and median raphe nuclei (DRN, MRN) for 5-HT neurons (Steinbusch, 1984; Hale and Lowry, 2011). Altogether, the structural properties of the monoaminergic neurons

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Abbreviations: 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, 5-hydroxytryptamine (serotonin); aCg, anterior cingulate cortex; aIns, anterior insular cortex; BLA, basolateral nucleus of the amygdala; CE, central nucleus of the amygdala; core, core of the nucleus accumbens; DA, dopamine; DLO, dorsolateral orbitofrontal cortex; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DOPAC, 3,4-dihydroxyphenylacetic acid; DRN, dorsal raphe nucleus; EDTA, ethylenediaminetetraacetic acid; HPC, hippocampus; HPLC, high pressure liquid chromatography; IL, infralimbic cortex; LC, locus coeruleus; LO, lateral orbitofrontal cortex; M2, motor cortex; MO, medial orbitofrontal cortex; MRN, median raphe nucleus; NA, noradrenalin; OFC, orbitofrontal cortices; pCg, posterior cingulate cortex; pIns, posterior insular cortex; PL, prelimbic cortex; shell, shell of the nucleus accumbens; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; VLS, ventrolateral striatum; VMS, ventromedial striatum; VTA, ventral tegmental area.

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favor a diffuse neurochemical influence that overlaps in many brain regions (Descarries et al., 1991; Descarries and Umbriaco, 1995).

The regulation of the neurochemical content for a monoamine in a brain region probably depends on local regulation that includes homologous and heterologous controls (Chesselet, 1984). In addition to the autoregulatory mechanisms exerted at cell body and terminal levels, monoamine content can be modified through indirect brain loops and network changes. For instance, lesioning approaches have led to the concept that cortical DA exerts an inhibitory control upon subcortical DA (Carter and Pycock, 1980; Pycock et al., 1980a,b) but these effects are somewhat controversial (Deutch, 1992; King and Finlay, 1997). Moreover, numerous lesioning experiments of brain regions or pharmacological experiments have reported combined changes of monoamine content in specific areas functionally interconnected (Chesselet, 1984; Soubrie et al., 1984; Millan, 2006; Schilman et al., 2010). These studies, however, barely inform on the principles of organization of monoaminergic systems. Thus, the existence of distal and neurochemical relationships within and between monoaminergic systems seems to be an important aspect for normal brain functioning but those site-specific interactions are largely unknown.

One possibility to address those monoaminergic relationships is to consider the existence of an inter-individual variability in the monoaminergic activities. Indeed, the neurochemical content for monoamines varies between individuals in a brain region. Thus, possible relationships and interactions in the neurochemical distributions of monoamines could be illustrated by specific correlations within and between brain regions among a large cohort of individuals.

We have examined the functional relationships between brain areas within a particular monoamine system network and between distinct monoaminergic systems in various brain areas involved in cognition. Tissue measurements were performed using a sensitive high-pressure liquid chromatography (HPLC) system coupled to electrochemical detection.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (n = 35; Charles River, Lyon, France) weighing 400–600 g were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12-light/dark cycle (dark from 8 p.m.) and had free access to water and food. All animals use procedures conformed to European Economic Community (86-6091 EEC) and the French National Committee guidelines (*décret* 87/848, Ministère de l'Agriculture et de la Forêt) for the care and use of laboratory animals and were approved by the Ethics Committee of Centre National de la Recherche Scientifique, Région Aquitaine-Limousin.

Tissue processing for histological verification and post-mortem analysis

Rats had been tested in several behavioral tests (decision-making, impulsivity, risk-taking) without any drug treatment. They were all tested in the same conditions, without any drug treatment or major stress. The more stressing test was the dark–light emergence task (10 min) that measures risk-taking through the choice for exploring a lightened compartment or a dark and protected one. This task was performed 4.5 months before rats were sacrificed and we can assume that the effect of the stress by this time was dissipated. Before the sacrifice, they were kept in the vivarium for 6 weeks without any testing. Rats were gently taken outside the vivarium to be immediately decapitated (within a few seconds) in a quiet room next door. Brains were removed rapidly, frozen using liquid nitrogen and stored until dissection at −80 °C. Dissection of brain areas was performed on a frozen microtome (Fig. 1). Bilateral punches of discrete regions were selected using a magnifying glass from frontal brain sections (250 μm) with stainless steel cannulae of 500 or 800 μm (anterior (aIns) and posterior insular cortex (pIns) only) inner diameter and pooled. Fig. 1 depicts the various areas sampled in the present study. In the case of the subthalamic nucleus (STN), tissue has been scarified using a blade around the STN in order to avoid the lateral hypothalamus (depicted in Fig. 1). The STN was removed after microtome cutting using a needle (right photomicrograph in Fig. 1). Most slices were imaged using a camera in order to determine a posteriori whether the selected areas were in a similar plane. Samples were stored in pre-weighed, small eppendorf tubes (0.6 ml volume) and stored at −80 °C. On the day of the biochemical analysis, after weighing the eppendorf, tissues were homogenized in 50 μl of 0.1 N $HClO_4$, sonicated, and centrifuged at 13,000 rpm for 30 min at 4 °C. Aliquots (20 μl) of the supernatants were injected into the HPLC system without dilution in the mobile phase.

Chromatographic analysis

Tissue concentrations of monoamines and their metabolites were measured by a sensitive HPLC-electrochemical detection (ECD) system. Samples were kept at 4 °C using an automated autosampler (Shimadzu, SIL-20A, Paris, France) and injected into the HPLC column (Hypersyl C18, 150 × 4.6 mm, 5 μm; C.I.L.-Cluzeau, Sainte-Foy-La-Grande, France) protected by a Brownlee–Newguard precolumn (RP-8, 15 × 3.2 mm, 7 μm; C.I.L.-Cluzeau). The mobile phase, delivered at 1.2 ml/min flow rate using a HPLC pump (LC20-AD, Shimadzu, France) was as follows (in mM): 60 NaH_2PO_4, 0.1 disodium EDTA, and 2 octane sulfonic acid plus 7% methanol, adjusted to pH 3.9 with orthophosphoric acid and filtered through a 0.22 mm Millipore filter. Detection of NA, DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT and its metabolite 5-hydroxyindole-3-acetic acid (5-HIAA) was performed with an amperometric cell Ag/AgCl (VT-03)