Supplementary Information

The material taught in the lecture course unit on the ANS

The general anatomical organisation and the process of neurotransmission in the ANS

Considering that anatomical considerations play a major part in the second- and third-semester classroom lectures preceding the fourth-semester physiology course, the course unit mainly focussed on aspects of neurotransmission in the two divisions of the ANS. In fact, general organisation and main anatomical differences were discussed within the first approximately 15 minutes, with the aim being to bring the ANS in general back into the students’ focus and give them the opportunity to ease their way back into the matter. Specifically, the following points were briefly addressed: firstly, the autonomic nervous system is divided into two anatomically and functionally distinct divisions, i.e., the sympathetic and the parasympathetic division. Secondly, in both systems, the efferent pathways of the ANS comprise two neurons that transmit their information from the central nervous system (CNS) to the effector organ. Thereby, the preganglionic neuron originates from the CNS, while the second neuron synapses with the former in the autonomic ganglion and directly innervates the effector tissue. The major learning objectives regarding this brief sub-section of the course unit are summarised in table 1.

The neurotransmitters and their receptors in the ANS

In order to further the students’ understanding of the process of neurotransmission in the ANS, the aspects of, firstly, ganglionic cholinergic (nicotinic) transmission, secondly, postganglionic adrenergic transmission and, thirdly, postganglionic muscarinic transmission, were discussed in detail over the course of approximately an hour. In the following section, these key topics are addressed and the main learning objectives outlined for each transmission scenario.
Ganglionic nicotinic neurotransmission. The ganglionic transmitter of both the sympathetic and the parasympathetic division is acetylcholine (ACh). ACh is synthesised in the ganglionic axon from choline (Ch) that is actively transported into the axon by means of a high affinity choline uptake transporter (HACU). ACh synthesis from its precursor involves the activity of choline acetyltransferase. Only, once ACh is placed into vesicles it can be actively released into the synaptic cleft following exocytosis. ACh binds to the ganglionic nicotinic ACh receptor (nAChR) that is equally sensitive to the exogenous neurotransmitter nicotine, while drugs like hexamethonium block that receptor. The action of ACh is terminated by the enzyme acetylcholinesterase (AChE) that resides in the cell membrane of the ganglionic cell.

Postganglionic adrenergic neurotransmission. Biosynthesis, reuptake and metabolism of the adrenergic transmitters, i.e., adrenalin and noradrenalin are briefly discussed. Specifically, noradrenalin is synthesised from tyrosine – that is built from phenylalanine in the liver – involving the activity of tyrosine hydroxylase, DOPA decarboxylase and dopamine hydroxylase. In the presence of (adrenal) N-methyl-transferase, noradrenalin is metabolised to adrenaline. In contrast, axonal reuptake and vesicular storage are mechanisms to end adrenergic transmission, as are methylation and deamination by catechol-O-methyl transferase and monoamine oxidase, respectively.

After this brief introduction into metabolic aspects, the receptors involved in adrenergic transmission are now discussed in detail. Hereby, the various receptor subtypes are presented with their downstream second messenger cascade that is activated following neurotransmitter binding. Specifically, activation of alpha1-receptors leads to the activation of phospholipase C (PL-C) that, in turn, causes an increase in IP3 levels. The subsequent release of Ca\(^{2+}\) from the sarcoplasmic reticulum, eventually, leads to vasoconstriction. Notably, this receptor subtype is mainly found in the smooth muscle surrounding blood vessels. Alpha2-receptors, in contrast, are negatively coupled to adenylate cyclase (AC) with their activation, therefore, leading to a decrease of cAMP. Moreover, alpha2-receptors are mainly auto-receptors that are
responsive for noradrenalin reuptake from the synaptic cleft. Furthermore, they are found on
Langerhans cells of the pancreas where their activation will lead to reduced secretion of
insulin. Activation of voltage-gated K⁺-channels leads to the inhibition of exocytosis and
secretion, while inhibition of voltage-gated Ca²⁺-channels results in the inhibition of secretion
in the gastrointestinal system.

As compared to alpha2-receptors, beta1-receptors are positively coupled to AC. Therefore,
their activation leads to an increase of cAMP. Notably, the closely linked phosphorylation of
L-type voltage gated Ca²⁺-channels results in Ca²⁺-influx and, hence, an increase of
intracellular Ca²⁺-levels that, in turn, provoke muscle contraction. Beta1-receptors are mainly
found in the juxtaglomerular apparatus of the kidney, where their activation provokes the
release of renin, and the heart, where they control heart rate, contractility and speed of
transmission. In contrast, activation of beta2-receptors (that are equally positively coupled to
AC) results in the activation of a Na⁺-/Ca²⁺-exchanger that, in turn, brings about the reduction
of intracellular Ca²⁺-levels and, therefore, smooth muscle relaxation. Given that these
receptors are located in the smooth muscle surrounding the respiratory tracts their activation
will lead to relaxation and a reduction of respiratory resistance. At the same time, beta2-
receptors are found in the blood vessels of the heart and in hepatocytes where their activation
furthers glycogenolysis.

Postganglionic muscarinergic transmission. Now following, the various subtypes of
mAChRs are introduced with the downstream second messenger cascades attributed to them.
Given that the relevant second-messenger cascades have been discussed in detail in the
preceding section, the lecture now rather summarises the various subtypes with respect to the
cascades involved and leaves more room for the presentation of the location and the actual
effects resulting from the activation of mAChRs. While mAChR M1, M3 and M5 subtypes
are positively coupled to PL-C, the M2 and M4 subtypes are negatively linked to AC. In
addition, some M1 and M2 receptors are associated with K⁺-channels. M1, M4 and M5
mAChRs are mainly found in neuronal cells of ganglia and CNS, whereas M2 receptors are predominantly located in the heart muscle, where their activation results in a decrease of the heart rate. In contrast, M3 receptors have been identified in cells of glandular organs, whose activity will increase upon receptor activation. Specifically, secretion of saliva, gastric juices and sweat is furthered by M3 mAChR activation, while the tone of gastrointestinal and urinary tract muscles increases.

The overall and specific functions of the ANS

While the functional differences between the two subdivision had been alluded to in various places already, the contrast between the two systems is elaborated in the following approximately 30 min long section of the lecture. Specifically, two slides listing the ergotrophic as compared to the trophotrophic effects summarise the ‘fight-or-flight’ as compared to the ‘rest-and-digest’ actions supported by the relevant division (table 2). Furthermore, a synopsis attributing the effect of the relevant division to the specific receptor sub-type in the single organ give the student the opportunity to think through the differences between the two systems (table 3). Finally, an emphasis is placed on the importance of the autonomic tone and the balance between sympathetic and parasympathetic activity, in that many sympathetic and parasympathetic neurons have specific spontaneous transmission frequency that ensures the balance between enhancing and decelerating neurons.

After a short break the students are then introduced to the case studies.
The case studies: answers and notes

Case study #1: organophosphate intoxication

Answers:

1. Organophosphates are irreversible inhibitors of AChE that will, therefore, cause a delay of the hydrolytic cleavage of ACh in the synaptic cleft, and, thus, lead to enhanced cholinergic transmission.

2. As a consequence, muscarinergic as well as nicotinic ACh receptors (mAChRs and nAChRs) are involved in the scenario to be discussed. This is why the symptoms expected are the following: increased salivation, lacrimation, urination, diaphoresis, and gastrointestinal motility.

3. Blockage of mAChRs by means of atropine reduces the effect of excessive amounts of ACh due to competitive inhibition of the receptors.

Notes:
The students needed some help to classify organophosphates correctly in that they were not known as AChE inhibitors; once informed about their use in chemical warfare and as insecticides the students were able to build on information they had gathered elsewhere.

Directly following the students’ presentation of their findings to the entire class, the symptoms were discussed in some more detail with respect to the following. Given that mAChRs are much more sensitive than nAChRs, muscarinergic effects of the intoxication are more prominent than those expected following activation of nAChRs. Specifically, nausea, diarrhoea, diaphoreses, miosis, salivation, bronchi-secretion and bronchi-constriction and bradycardia are to be expected. Among the nicotinic effects muscle weakness and twitches are expected, while anxiety, headaches, cramps and respiratory paralysis are considered as central effects of an organophosphate intoxication. Atropine given intravenously is used to achieve a normalisation of the vegetative functions, but measures to prevent further inhalation and reabsorption are necessary as well as the symptomatic treatment of cramps using muscle
relaxants. Importantly, ganglionic intervention is not desirable when considering that blocking agents would provoke numerous and complex effects since both the sympathetic and the parasympathetic division of the ANS are being influenced.

Case study #2: atropine intoxication

Answers:

1. Atropine is an inhibitor at the mAChR that competitively inhibits ACh from exerting its effect.

2. Therefore, the regulation of vegetative functions through the balance between the two divisions of the ANS is impaired in that it is shifted towards a regulation via the sympathetic division, since the parasympathetic dampening influence on the control of physiological processes is blocked at the mAChRs.

3. This is why redness in the face and dryness of the mucosa are observed. In the same vein, difficulty to swallow occurs and the heart rate is increased. Mydriasis is noticed as well as hyperthermia.

Notes:
The students had less difficulty to work on this case study, probably due to the fact that intoxication with Atropa belladonna was known as a not infrequent occurrence among small children. However, it proved useful to point the students to the importance of identifying the receptors and messenger cascades involved in the pathology in order to correctly explain the symptoms they had identified.

Following the students’ presentation to the class, the subsequent aspects were expanded on further: in the first place, the effect of atropine roots in the actual prevention of the parasympathetic contribution to the regulation of vegetative function. At this point, the table already displayed and discussed during the theoretical part of the course was projected again and the antagonistic effects of the two divisions of the ANS were pointed out (table 2). In this
context, the tutor pointed the students to the fact that the apparent antagonism between the two divisions was not given on a one-to-one basis in that innervations of various organs was only of sympathetic nature. Furthermore, it was clearly delineated which effects followed from parasympathetic blockade as compared to sympathetic ‘activation’. To conclude, additional measures to treat intoxication were discussed, i.e. the use of physostigmine intravenously as well as active coal.

Case study #3: Raynaud’s phenomenon

Answers:

1. The receptors mainly involved in this clinical picture are alpha1-receptors whose activation will finally lead to vasoconstriction. The second-messenger cascade being triggered following the activation of the alpha1-receptor involves the activation of PL-C, a subsequent increase of IP3 and the resulting Ca\(^{2+}\)-efflux from the sarcoplasmic reticulum.

2. Blockage of alpha1-receptors is a therapeutic approach to be considered.

3. Key collateral effects are the feeling of a blocked nose and sickness. Furthermore, impairment of orthostatic regulation due to the vasodilatation following the block of alpha1-receptors in the periphery might be expected.

During the discussion within the team the tutor needed to point out that sympathetic intervention was the therapeutic approach of choice considering that the cause of the symptoms described was clearly identified as sympathetic impairment. In fact, students came up with the idea to further muscarinergic transmission in the periphery in order to counteract the pathologically enhanced sympathetic activity. Here, the tutor took – again – the opportunity to specifically emphasise and discuss that the two divisions of the ANS do not represent ‘one-on-one antagonists’.
Case study #4: Sympathetic nervous system and blood pressure

Answers:

1. Both alpha- and beta-receptors are involved in the regulation of blood pressure. In particular, alpha1-receptors are responsible for the regulation of vasoconstriction in the periphery, while beta1-receptors are located in the heart muscle where they mediate effects on heart rate (positive chronotrope effect), contractility (positive inotrope effects) and transmission velocity (positive dromotrope effects).

2. From a therapeutic point of view, alpha1- as well as beta1-receptor antagonist could be used. These prevent noradrenalin from exerting its effect on the relevant receptors, i.e., vasoconstriction in the periphery is inhibited, which, eventually, leads to a decrease of peripheral resistance. At the same time, beta1-receptor antagonists decrease the heart rate as well as the (unwanted) contractility of the heart muscle.

3. Collateral effects concerning alpha1-receptor antagonists are similar to those presented in case study #3 in that impairment of orthostatic regulation due to the vasodilatation following the block of alpha1-receptors in the periphery may be a consequence of these drugs. In contrast, collateral effects of beta1-antagonist might result from their lacking selectivity.

4. A partial block of beta2-receptors may lead to the obstruction of the respiratory tract and, hence, lead to increased respiratory resistance. Therefore, beta1-receptor antagonists should not be given to patients who are at risk of developing asthma.